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Studies on Carbohydrate-derived Hydroxamic Acid Inhibitors of the Bacterial Enzyme LpxC

by

Xuechen Li

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfilment of the requirements for the degree of

Master of Science

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "**Studies on Carbohydrate-derived Hydroxamic Acid Inhibitors of the Bacterial Enzyme LpxC**" in partial fulfillment of the requirements for the degree of **Master of Science**.

Dr. Ole Hindsgaul (Supervisor)

Dr. Rik R. Tykwinski (Chair)

Dr. Mark S. Peppler

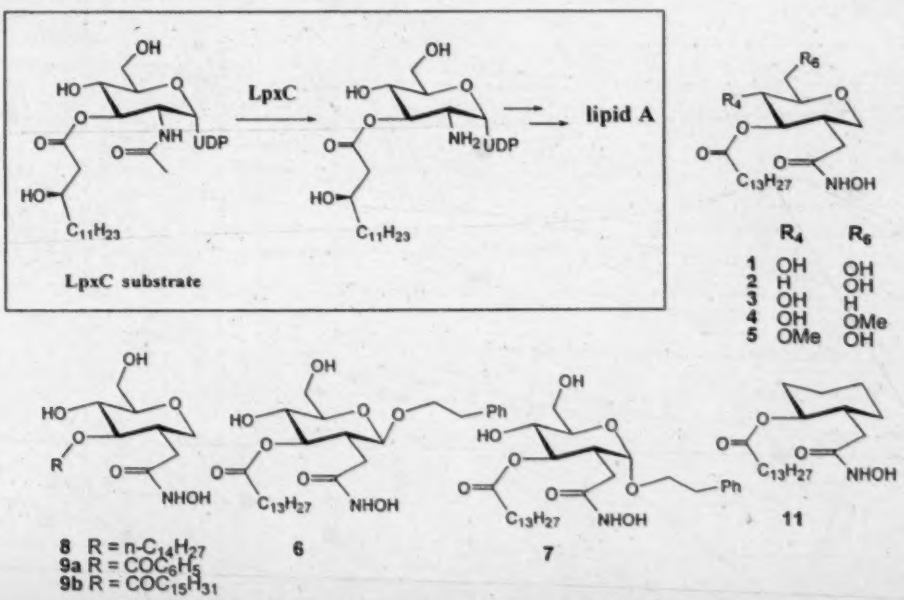
Dated: June 9, 2003

ABSTRACT

Recently, the biosynthetic pathway of lipid A has been recognized as a promising new target for the development of antibiotics with novel antibacterial action against Gram-negative bacteria. Of many enzymes involved in lipid A biosynthesis, the *N*-deacetylase named LpxC, which catalyzes the first committed step in the pathway, has been suggested to be a good pharmaceutical target.

A class of carbohydrate-derived hydroxamic acid, developed in our laboratory, was previously shown to exhibit a wide spectrum of inhibitory activity. This class of LpxC inhibitor contains the most potent compound (**1**) reported to date that inhibits the enzyme from most diverse Gram-negative bacteria with an IC_{50} = 7.0 ± 0.5 μM (*A. aeolicus* LpxC); 7.2± 1.9 μM (*E. coli* LpxC).

In order to gain a more detailed understanding on a molecular level of the LpxC-inhibitor complex, as well as to establish a SAR (structure-activity-relationship), a chemical mapping approach was chosen. Ten analogs of **1** have been chemically synthesized and enzymatically evaluated.



Evaluation of the analogs with LpxC (*E. coli*) indicated that the two hydroxyl groups of the GlcNAc residue are involved in the binding interaction with the enzyme, and certain long acyl chains, neither too short nor too long, are crucial for the inhibition. Replacement of the myristoyl ester chain with a benzoyl group did not diminish inhibitory activity. Phenylethyl groups could not substitute for UDP of the native substrate.

During the course of these studies, inhibitor **1** was resynthesized in quantities that permitted NMR studies at 800 MHz to define for the first time the three-dimensional structure of the LpxC/**1** complex in solution.

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I dedicate this to the memory of my Grandfather and beloved Uncle who passed away during my studies.

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List of Abbreviations

Ac	acetyl
AIBN	2'2-azobisisobutyronitrile
aq.	aqueous
b	broad
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
calcd	calculated
d	doublet
DAST	diethylaminosulfurtrifluoride
DBMP	2,6-di(tert-butyl-4-methyl) pyridine
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
EDC	1-ethyl-3(3′ dimethylaminopropyl)carbodiimide-HCl
ES HRMS	electrospray high resolution mass spectrometry
equiv.	equivalent
GCOSY	gradient coupling correlated spectroscopy
Glc	glucose
GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-glucose
HMQC	heteronuclear multiple quantum coherence
Hz	hertz

J	coupling constant
IC_{50}	inhibitor concentration required to give 50% inhibition
Me	methyl
mg	miligram(s)
MHz	megahertz
ml	milliliter(s)
mol	mole(s)
mmol	millimoles(s)
M.S.	mass spectrometry or molecular sieves
NOE	nuclear overhauser effect
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
PyBOP	benzotriazol-1-yloxy tripyrrolidinophosphonium hexaflurophosphate
Pyr	pyridine
q	quartet
s	singlet
satd.	saturated
t	triplet
TBAF	tetrabutylammonium fluoride
TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsiyl
TEA	triethylamine

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
p-TSA	*p*-toluenesulfonic acid monohydrate
UV	ultraviolet

Chapter 1

Introduction

1.1 Bacteria

Bacteria are a type of prokaryote. Bacteria have a single circular DNA chromosome found within the cytoplasm of the cell as they do not have a nucleus. Many bacteria can cause human and animal disease. Historically, bacteria have been classified as either Gram-negative or Gram-positive. Gram-negative and Gram-positive bacteria are distinguished according to whether or not they take up the Gram stain (a procedure developed in 1884 by Christian Gram in which heat fixed cells are successively treated with the dye violet and iodine and then destained with either ethanol or acetone) [1]. Gram-positive bacteria include *Streptococcus, Staphylococcus, Enterococcus, Listeria monocytogenes, Bacillus anthracis* and *Actinomycetes,* while Gram-negative bacteria include *Escherichia*, *Pseudomonas*, *Salmonella*, *Shigella* and *Vibrio.*

The inherent factor that produces the difference in Gram-staining derives from difference in composition and construction of the cell wall. Both Gram-positive and Gram-negative bacteria have an inner cell envelope made up of peptidoglycan (murein) and a phospholipid bilayer. Gram-negative bacteria have a thinner peptidoglycan than Gram-positive bacteria. However, they have a unique outer membrane, which is rich in a unique biopolymer, termed lipopolysaccharide (LPS) (Figure 1.1) [2]. LPS is often referred to as an endotoxin, since it can trigger a cascade of events, including high fever and a drop in blood pressure. The Gram-negative outer membrane blocks antibiotics,

dyes and detergents, thus protecting the sensitive inner membrane and the cell wall. Therefore, Gram-negative cells are resistant to lysozyme and penicillin whereas Gram-positive cells are vulnerable to them.

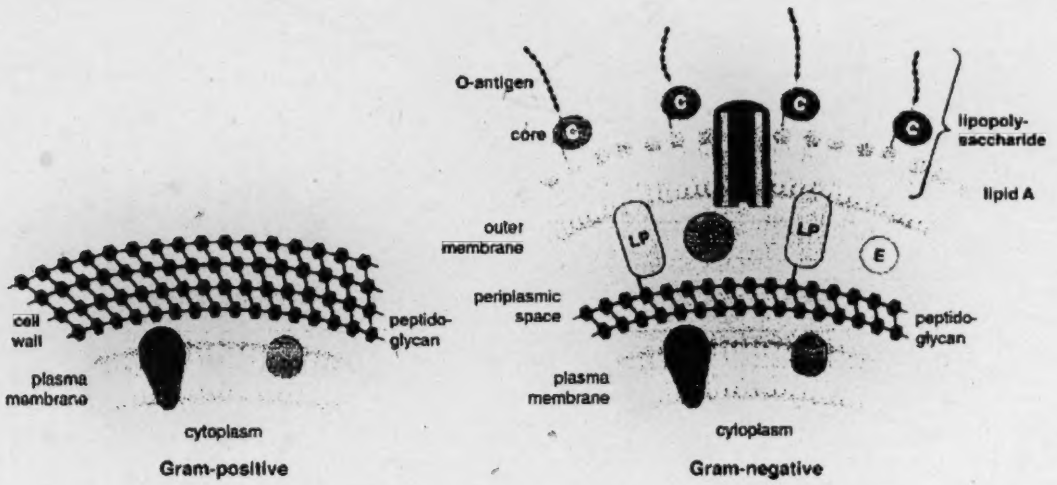


Figure 1.1. The cell envelope of Gram-positive and Gram-negative bacteria [3]. The purple cylinder in the Gram-negative bacteria picture represents a porin through which small molecules like antibiotics can enter the bacteria

1.2 Antibiotics

1.2.1 Introduction

In the treatment of infectious diseases, antibiotics are natural or synthetic substances which can kill bacteria or inhibit the growth, without doing much harm to the treated individuals. Screening of natural product extracts that can kill bacteria and

subsequent semisynthetic modifications of lead compounds have been the main historical means to produce antibiotics. For instance, pencillins were first found in fungi and semisynthetic modification resulted in ampicillin and amoxycillin [4].

1.2.2 Common antibiotics and their targets

The mode of action of antibiotics depends on their target, and there are four main classes [3, 4]:

1) antibiotics that target the cell-wall biosynthesis (peptidoglycan): e.g., β-lactams (penicillins), glycopeptides (vancomycin)

2) antibiotics that target protein biosynthesis: e.g., aminoglycosides (streptomycin), macrolides (erythromycin), tetracyclines (tetracycline), lincomycins (clindamycin)

3) antibiotics that target DNA replication and repair: e.g., fluoroquinlones

4) antibiotics that act on cell membranes: e.g., gramicidin [5]

1.2.3 Antibiotic resistance

Antibiotic resistance has become a big public health issue. Bacteria are living organisms, and they adapt quickly to external stress. Therefore, in the war against bacterial disease, we are in danger of lacking antibiotics to treat bacterial infections, since the rate of increase of bacterial resistance is extremely high. Bacteria can develop resistance to antibiotics after treatment, and can pass genes for multiple drug resistance between strains and even between species. Once an antibiotic enters widespread human

therapeutic use, its effective life span becomes limited. For example, penicillin became generally available for treatment of bacterial infections, especially those caused by staphylococci and streptococci, in 1946. Initially, the antibiotic was effective against all sorts of infections caused by these two Gram-positive bacteria. Resistance to penicillin in some strains of staphylococci was recognized almost immediately. Another example is Vancomycin, which is considered to be the last resort for defense of certain bacterial infections. Since 1989, vancomycin-resistant enterococci (VRE) have spread around the world [6]. Bacterial resistance to antibiotics is generally just a matter of time.

Three basic mechanisms of antibacterial resistance have been proposed [4]: 1) active transport of antibiotics out of the microbe [7]; 2) modification or metabolism of the antibiotic molecule [8]; 3) reprograming of the target structure [9], for example, mutations from the D-Ala-D-Ala to D-Ala-D-lactate lead to the inability of Vancomycin antibiotics to bind.

The market for new antibiotics is picking up again after a short break [10]. More and more companies and academic research groups are turning their attention to this war against bacteria [11], and although new antibiotics are being developed, most of them are improved versions of existing drugs exerting their action on classic targets. To circumvent existing resistance mechanisms, the search for compounds against new bacterial targets with novel antibacterial action is urgently needed.

1.2.4 Carbohydrate-based antibiotics

Carbohydrates are important structural building blocks of cells and components of numerous metabolic pathways [12, 13]. For this reason, many existing antibiotics are often based on sugar moieties or contain a glycan portion in their structure which is essential for inhibitory activity, such as Streptomycin and Vancomycin (Figure 1.2). Also, some antibiotics that are non-carbohydrate molecules target an enzyme or receptor associated with carbohydrate synthesis, metabolism or recognition.

Carbohydrate-based antibiotics have been attracting increasing attention in recent years. In 1999 and 2001, two review papers (1)" Carbohydrate-Based Antibiotics: A New Approach to Tackling the Problem of Resistance" [3] and (2) "Carbohydrate Mimetics: A New Strategy for Tackling the Problem of Carbohydrate-Mediated Biological Recognition" [14], have summarized the design strategies and collected many examples of potent carbohydrate-based antibiotics that are in their early stages of development.

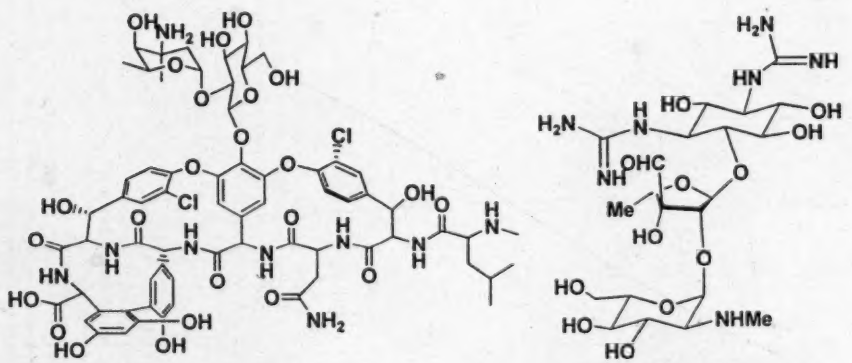


Figure 1.2. Two major carbohydrate-based antibiotics: Vancomycin and Streptomycin

1.3 LPS – An attractive new target to develop antibiotics against Gram-negative bacteria

1.3.1 Introduction

A new promising target is the unique lipopolysaccharide (LPS) part of Gram-negative bacteria [10]. As mentioned above, LPS is the major structural constituent of the outer membrane. Currently no marketed antibiotics are directed against the LPS biosynthetic pathway. Another attractive feature of LPS as a target is drawing much attention due to its biological mechanism of toxicity. LPS is also known as endotoxin [15]. Upon treatment of higher animals and humans with conventional antibacterial agents, LPS is released from the dying bacteria causing septic shock. The annual number of deaths from septic shock is about 70,000 in the USA alone [16], a figure comparable to AIDS. The inhibition of the biosynthesis of LPS is bacteriocidal or bacteriostatic and is also expected to reduce the amount of endotoxin released when other antibiotics are administered simultaneously. Therefore, an inhibitor of LPS biosynthesis would have multiple beneficial effects for the therapy of the Gram-negative infections.

LPS consists of lipid A, two KDO residues, the core oligosaccharide and the O-antigen (Figure 1.3) [17, 18]. The lipid A and two KDO units have been shown to be minimally required for the bacterial growth in general [19, 20].

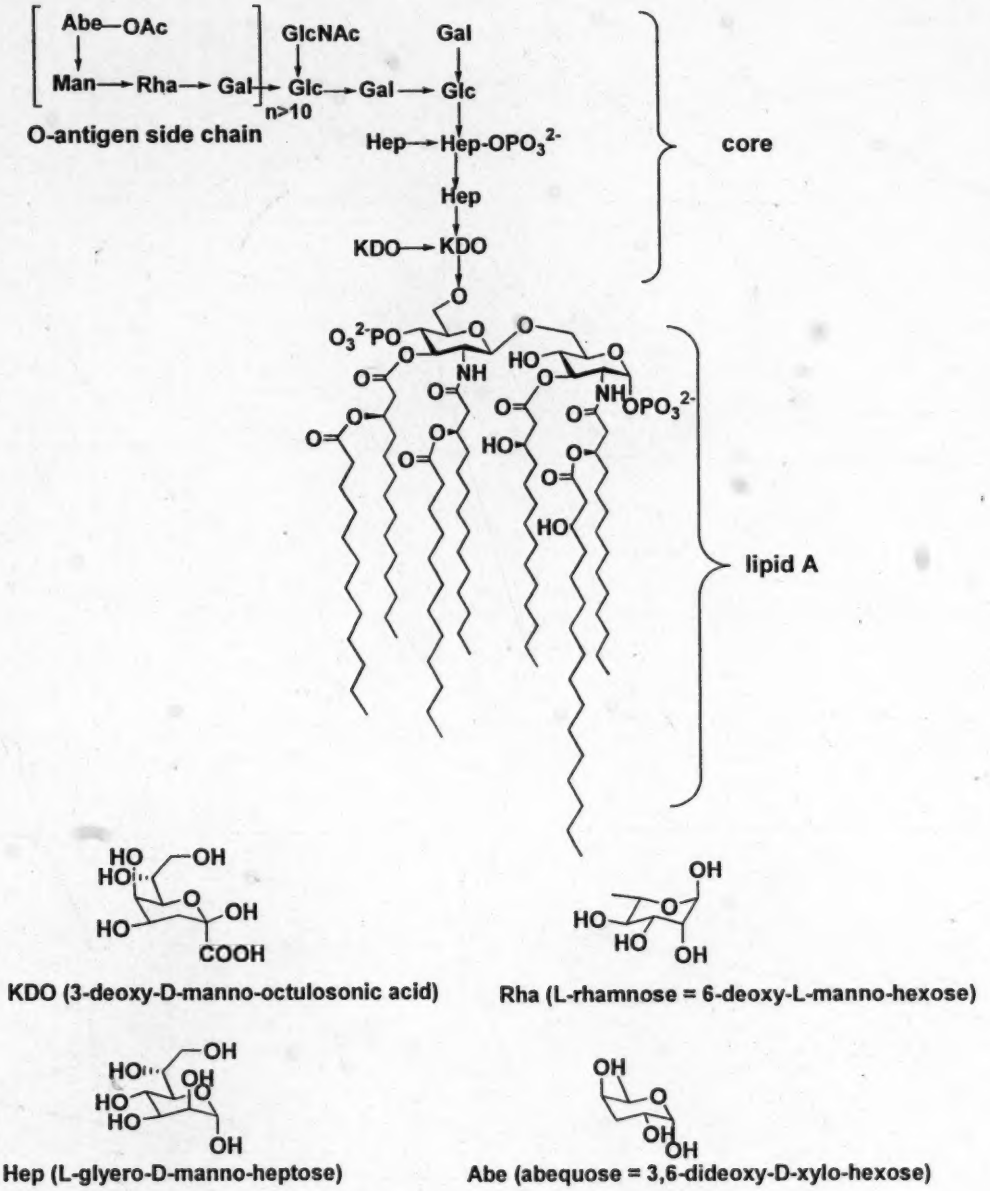


Figure 1.3. The structure of a typical lipopolysaccharide (LPS)

The first report on the inhibition of LPS biosynthesis was targeting the enzyme CMP-KDO synthetase to block the formation of KDO units of LPS. Some compounds

(Figure 1.4) are found to be very potent inhibitor *in vitro*. However they did not lead to useful therapeutics [21].

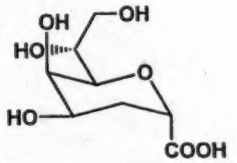


Figure 1.4. An inhibitor targeting CMP-KDO synthetase

1.3.2 Lipid A as a new target

Another essential part of LPS, lipid A, has been investigated in recent years. Lipid A, the hydrophobic anchor of LPS, is a glucosamine disaccharide-based phospholipid. The enzymology and molecular genetics of lipid A biosynthesis in *E. coli* have been elucidated in the last decade (Figure 5) [19, 22, 23, 24]. Many Gram-negative bacteria, including most pathogens, synthesized lipid A species resembling the one found in *E. coli*. [19, 20]

Many enzymes involved in the biosynthesis of lipid A provide good targets for the development of new antibacterial agents and, of these, LpxC has been extensively studied. LpxC is a *N*-deacetylase (Figure 1.5), highly conserved with homologues in more than 40 Gram-negative species [25]. Recent studies have shown that LpxC, a metalloenzyme, uses a single Zn^{++} ion to catalyze deacetylation [26] (Figure 7). The zinc ion-dependent mechanism of LpxC makes it attractive as a target, as many analogous

zinc metalloproteinases have been described, such as angiotension-converting enzyme (ACE) [27, 28], and endothelin-converting enzyme (ECE) [29, 30].

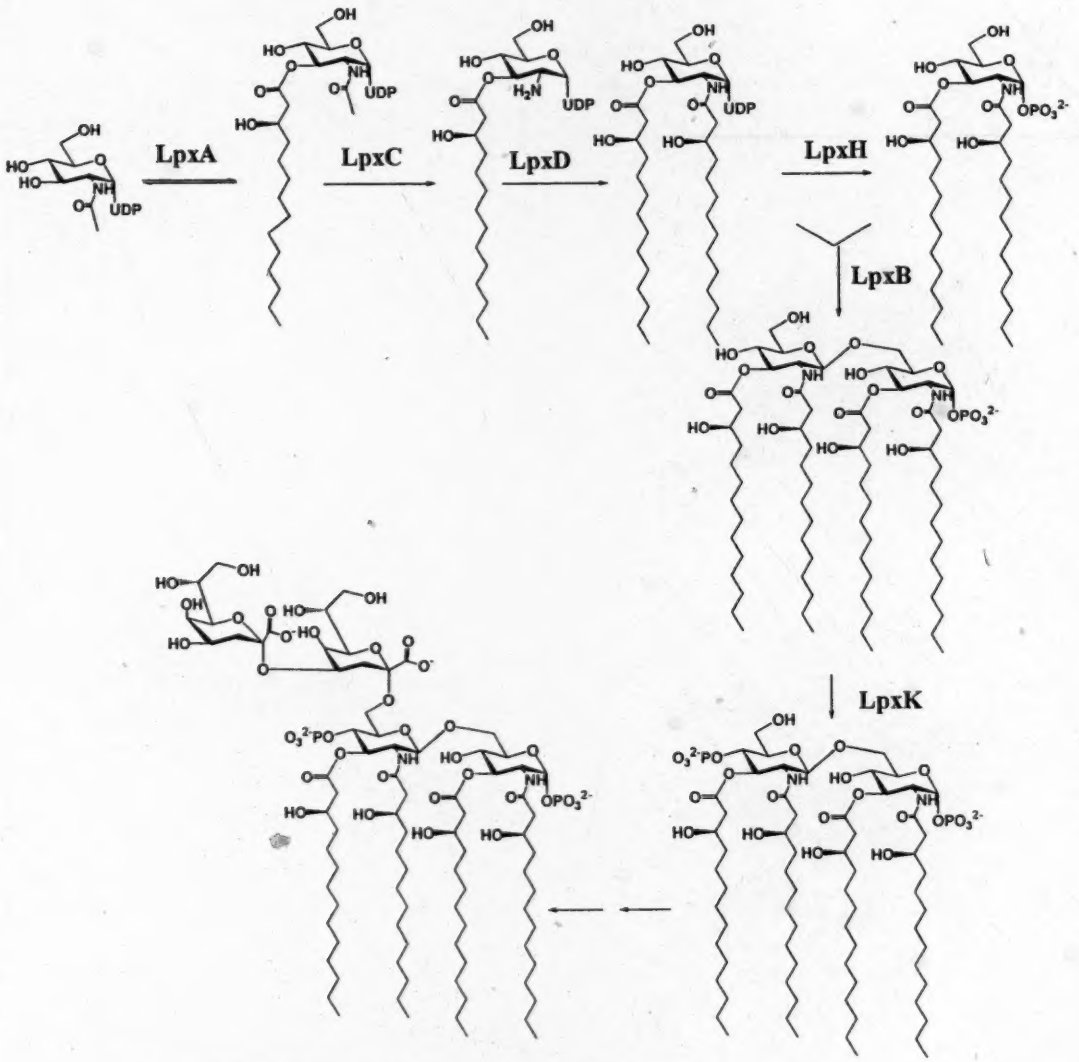


Figure 1.5. Biosynthesis pathway of lipid A

1.3.3 Current inhibitors targeting the biosynthetic pathway of lipid A

In 1996, after screening and subsequent optimization [31], Merck scientists reported hydroxamic acid derivatives (Figure 1.6) that were potent inhibitors of LpxC. The limitation of this class of LpxC inhibitor is that they are only effective against *E. coli*. Extensive series of analogues failed to yield compounds that were broad spectrum inhibitors [32, 33, 34, 35]. More recently, another class of hydroxamic acid, namely sulfonamide derivatives of the α-(*R*)-amino hydroxamic acid derivatives, has been reported to show antibacterial activities against a wider panel of pathogens [36].

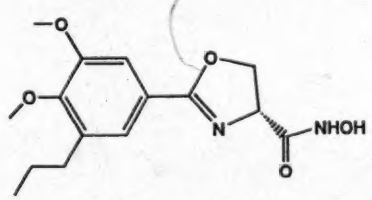


Figure 1.6. A Merck inhibitor targeting LpxC

LpxC requires enzyme bound Zn^{++} to effect amide bond cleavage. The well-know affinity of hydroxamic acids for zinc has led to the proposal that the inhibitors act by coordinating to the single catalytic zinc ion [26, 37]. Our laboratory conceived of the idea of incorporating the hydroxamic acid into the substrate at the position of the acetamide group undergoing enzymatic hydrolysis. Compound **1** was prepared and has shown to exhibit a wide spectrum of inhibitory activity with an IC_{50} = 7.0 ± 0.5 μM (*A. aeolicus* LpxC); 7.2 ± 1.9 μM (*E. coli* LpxC) (Figure 1.7) [38]. This class of LpxC inhibitors contains the most potent compounds in literature so far that inhibits the LpxCs of the most diverse Gram-negative bacteria. The proposed hypothetical complex with **1** is schematized below (Figure 1.7).

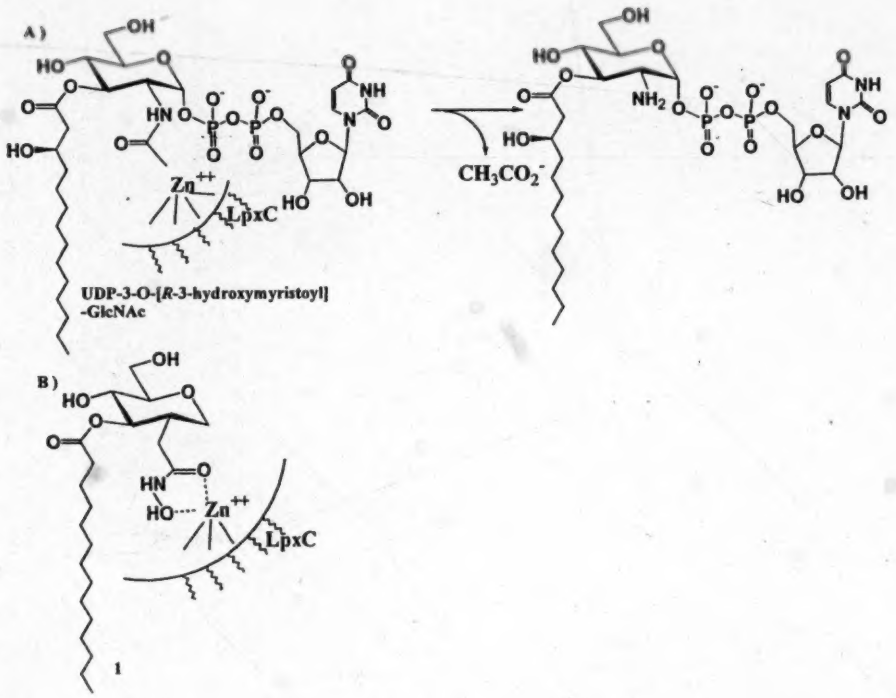


Figure 1.7. A) The reaction catalyzed by LpxC. B) Proposed mode of binding of inhibitor **1** to LpxC

However, despite of their potent activity *in vitro* against LpxC, the carbohydrate-based inhibitors of LpxC are not very active as antibacterial agents. The probable reason is that these compounds cannot cross the cell membranes and enter the cells to access the enzyme.

1.4 Scope of the thesis research project

In order to gain a more detailed understanding on a molecular level of the LpxC-substrate-based inhibitor complex, as well as to establish to a SAR (structure-activity-relationship), a chemical mapping approach employing single hydroxyl-modified analogs of **1** was chosen. That is, one at a time, each of the hydroxyl groups of **1** was replaced by a hydrogen or methoxy group to map the hydrogen-bonding pattern of the active sites (Figure 1.8). A deoxy derivative can provide an assessment of the corresponding hydroxyl's contribution for a hydrogen-bonding interaction, while a methoxy derivative can yield information about steric tolerance of the protein at the active site.

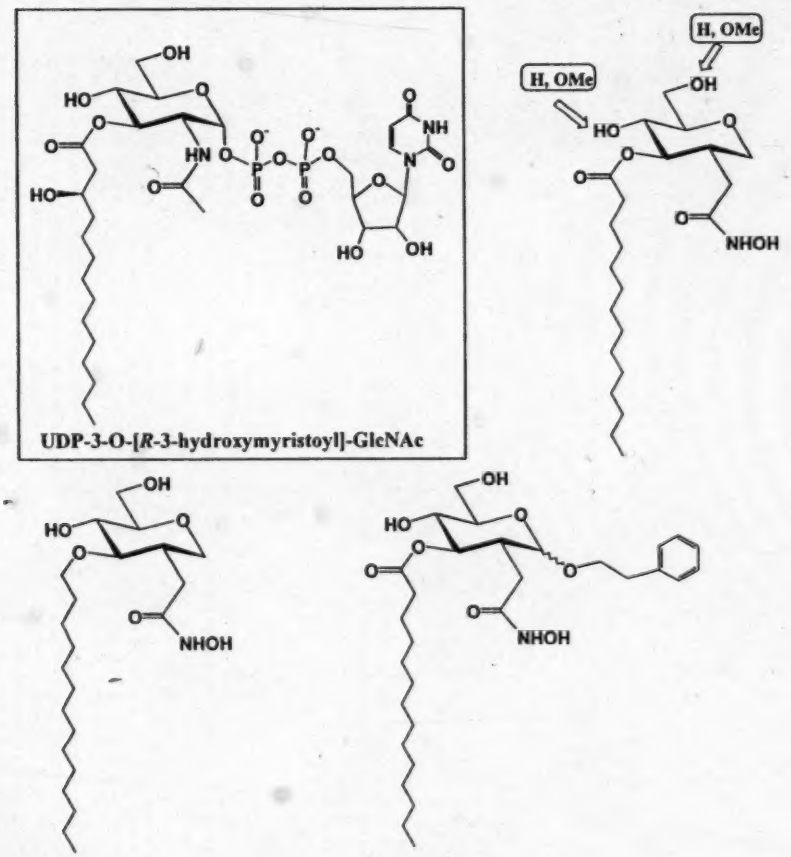


Figure 1.8. General synthetic targets for SAR studies

Since the natural substrate contains a UDP moiety at C-1, we also prepared both an α and a β O-glycoside at C-1 to probe whether replacement of UDP with neutral groups would be tolerated.

Additionally, since the ester group at OH-3 might be metabolically and synthetically unstable, we replaced it with an ether long-chain. This would facilitate synthesis and would produce a more stable drug candidate, if this modification is tolerated.

All of analogs above would have altered hydrophobicity as well, and might begin to overcome the impermeability problem.

Chapter 2

Chemical Synthesis

2.1 Introduction and synthetic strategy

This chapter describes the chemical synthesis of the lead compound (**1**), a known LpxC inhibitor, and its analogs (Figure 2.1).

To the best of our knowledge, there are no examples reported in literature of the synthesis of a 1,2-dideoxy-2-C-branched-chain-like sugar starting from a carbohydrate substrate, so this was envisaged as one of the key synthetic challenges. We found that 1,6-anhydro-β-D-glucopyranose underwent reduction with Et_3SiH in the presence of a Lewis acid, providing rapid access to carbohydrate synthons of this type (Scheme 2.1). This process proved to be very efficient. We anticipate that this approach will find broad utility in the synthesis of new non-natural carbohydrates.

A second potential problem is the presence of both an ester group and a hydroxamic acid in the final compound. Introduction of a hydroxamic acid is usually accomplished by coupling a carboxylic acid with *O*-benzylhydroxylamine hydrochloride ($BnONH_2 \cdot HCl$) to form the carboxy *N*-benzyloxyamide (CONHOBn) group. The nitrogen in this group can potentially undergo *N*-acylation and *N*-alkylation, typically with reagents like Ac_2O/pyridine and MeI/NaH. Once the ester group and CONHOBn are installed, further transformation would then likely be very tricky. Therefore the strategy that evolved was to introduce the ester and CONHOBn groups as near to the end as possible.

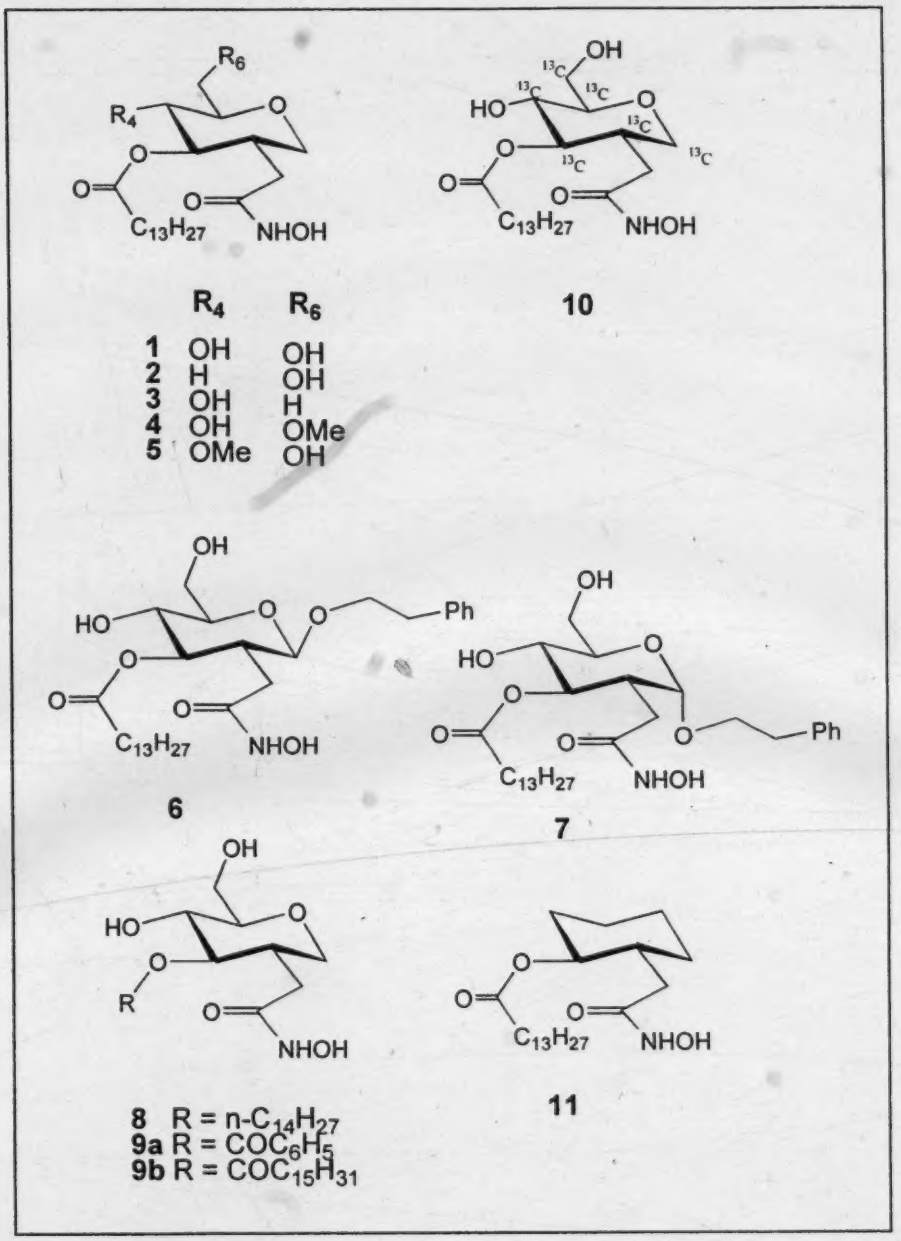
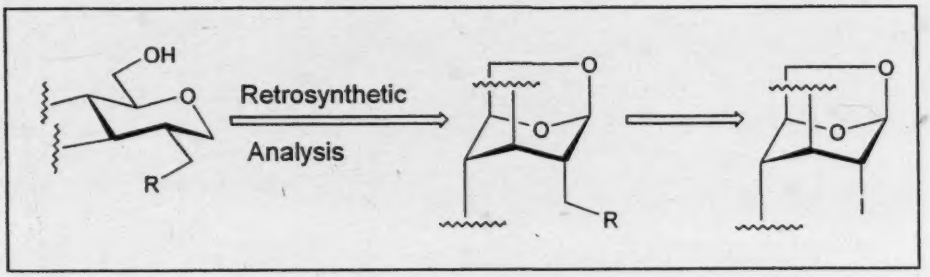


Figure 2.1. General structure of the synthetic targets



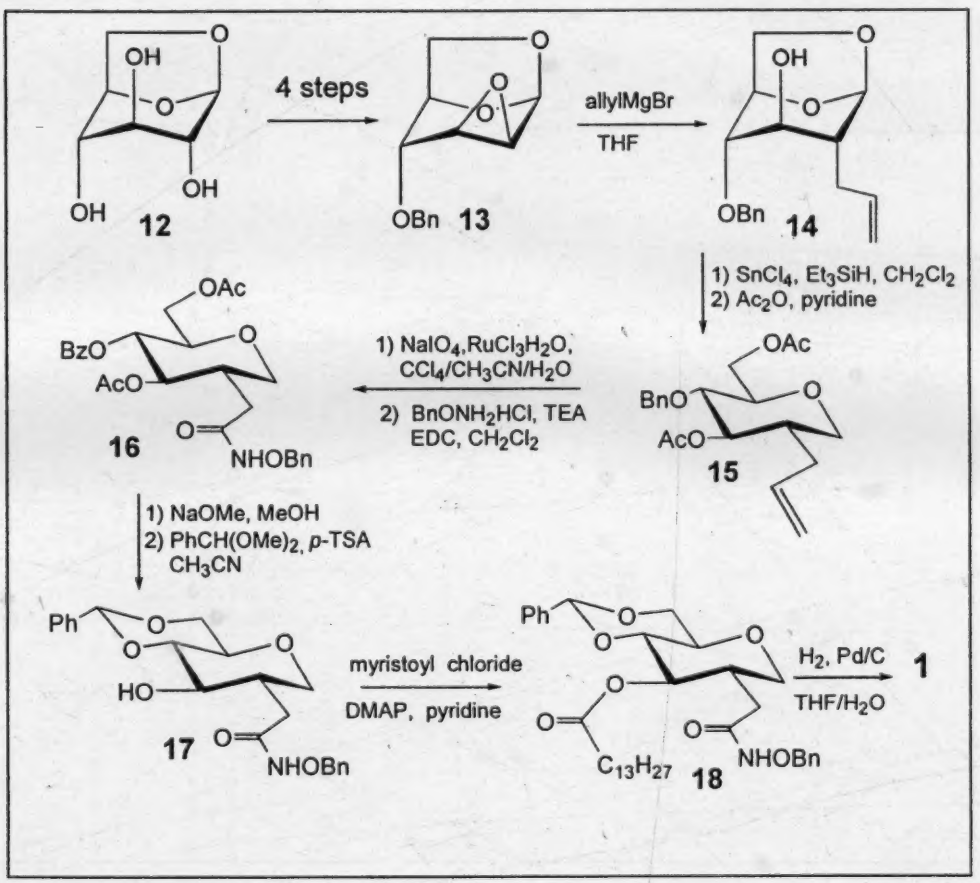
Scheme 2.1

2.2 Chemical Synthesis

2.2.1 Preparation of compound 1 by two different synthetic routes

Two synthetic routes were evaluated for the preparation of LpxC inhibitor **1**. Dr. Taketo Uchiyama in this laboratory developed the first route, which had not been published. In his procedure (Scheme 2.2), "Cerny epoxide" **13**, available from 1,6-anhydro-D-glucose in four steps [39], was opened with allylmagnesium bromide to give the expected *trans*-diaxial product **14** (79%). Reductive opening of the 1,6-anhydro ring of **14** with $SnCl_4$ and Et_3SiH followed by *O*-acetylation (Ac_2O/pyridine) gave **15** (88%). Oxidative cleavage of the double bond [40] in **15** with ruthenium tetroxide (generated in situ from $RuCl_3$ and $NaIO_4$) gave a carboxylic acid derivative with concomitant oxidation of the benzyl protecting group at OH-4 to a benzoate group. The crude product was reacted with *O*-benzylhydroxylamine hydrochloride and a coupling reagent (1-ethyl-3(3'dimethylaminopropyl)carbodiimide-HCl, EDC) to give the protected hydroxamic acid derivative **16** (76%). Deacylation (NaOMe) of **16** to remove the *O*-acetyl group and

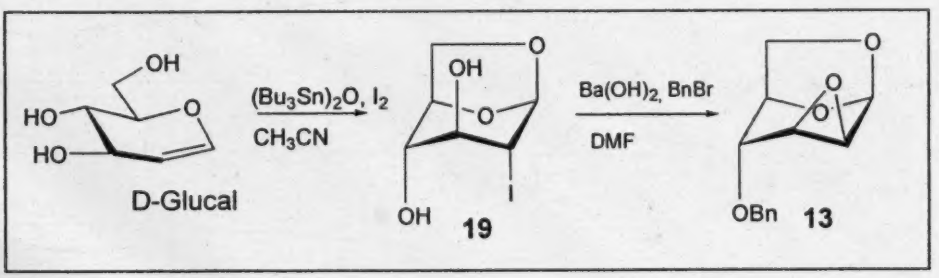
the newly formed benzoate group was followed by reaction with benzaldehyde dimethylacetal under acidic catalysis to give **17** (64%). *O*-Acylation with myristoyl chloride then gave **18** (52%). The low yield of **18** in the *O*-acylation step was attributed to concomitant *N*-acylation. Deprotection of **18** (H_2, Pd/C, THF/H_2O) gave the final product **1** (45%).



Scheme 2.2

While we repeated this synthetic route to prepare **1** for further study, and to collect missing spectral data for the intermediates, some improvements were made as described below.

Firstly, "Cerny expoxide" **13** was produced from D-glucal in two steps [41] instead of four steps (Scheme 2.3). Compound **19** was prepared according to the literature [42, 43]. Roush and coworkers recently reported that the benzylation-cyclization of **19** could be accomplished with $Ba(OH)_2$ and BnBr in DMF to give expoxide **13** in 82% yield [41], but the experimental details were not provided. However, we found that reaction conditions were important for this transformation, the yields being sensitive to scale, reaction concentration and reaction time. However, reasonable yields (50-70%) could be obtained on 1.5-6.0 gram scales. Despite these difficulties, this route is still better than the 4-step "Cerny expoxide" **13** synthesis (30%) (Scheme 2.2) in overall yield

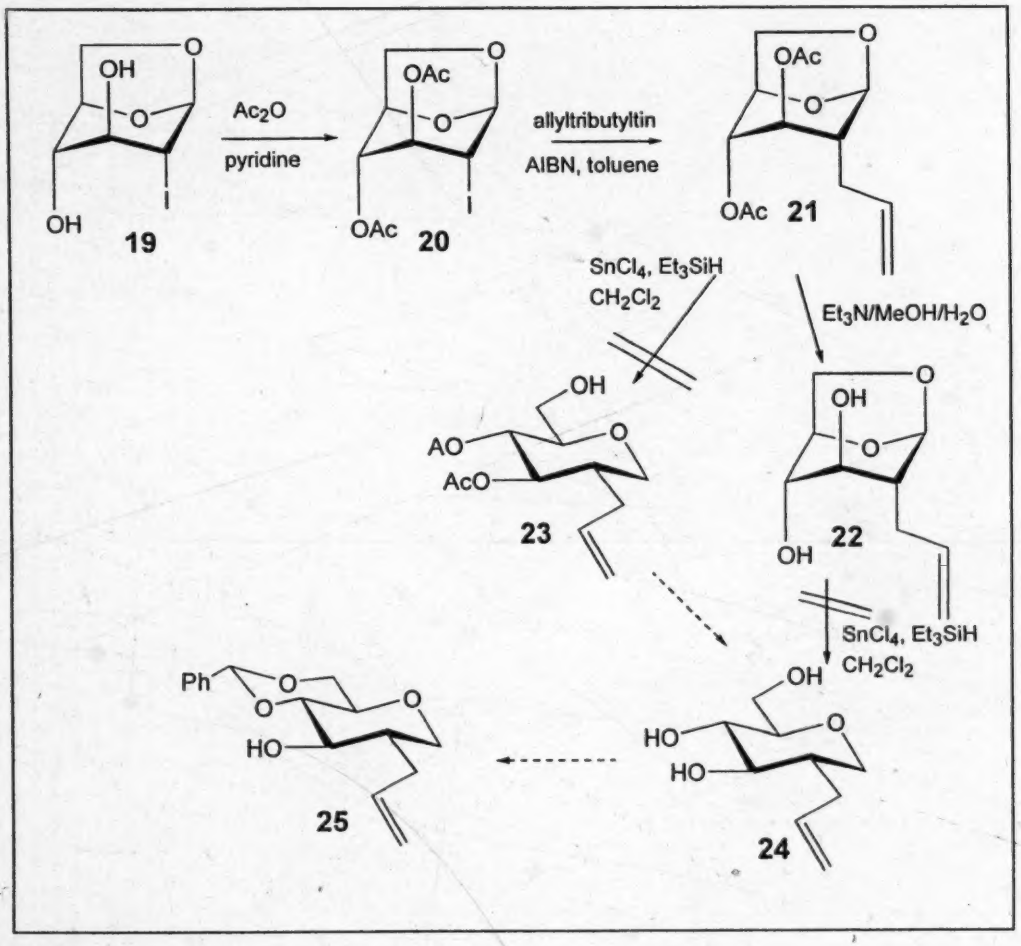


Scheme 2.3

Secondly, hydrogenation (H_2, Pd/C) of **18** in AcOH gave better yields (71%) than in THF/H_2O (45%) and the reaction time was reduced to 6 hours from 16 hours.

Some drawbacks with the improved Uchiyama synthetic scheme were identified. The synthesis is not practical on a large scale. For instance, in the reaction to produce **14**, the yield of byproduct (1,6-anhydro-4-O-benzyl-2-deoxy-β-D-glucopyranoside) increased with the reaction scale, and the cleavage of a benzyl group of **14** occurred during reductive opening of the 1,6-anhydro ring with $SnCl_4$ and Et_3SiH. Furthermore, the solubility of compound **17** is very poor in most chromatography solvents, which resulted in difficulties in its purification. Recrystallization was also not an effective means of purification. As a result, the yields were only around 50-60%. The early introduction of the CONHOBn groups created problems with a side reaction involving the nitrogen at the *O*-acylation stage which will be further discussed later for the analogue synthesis. Therefore, a new synthetic pathway to **1** was developed.

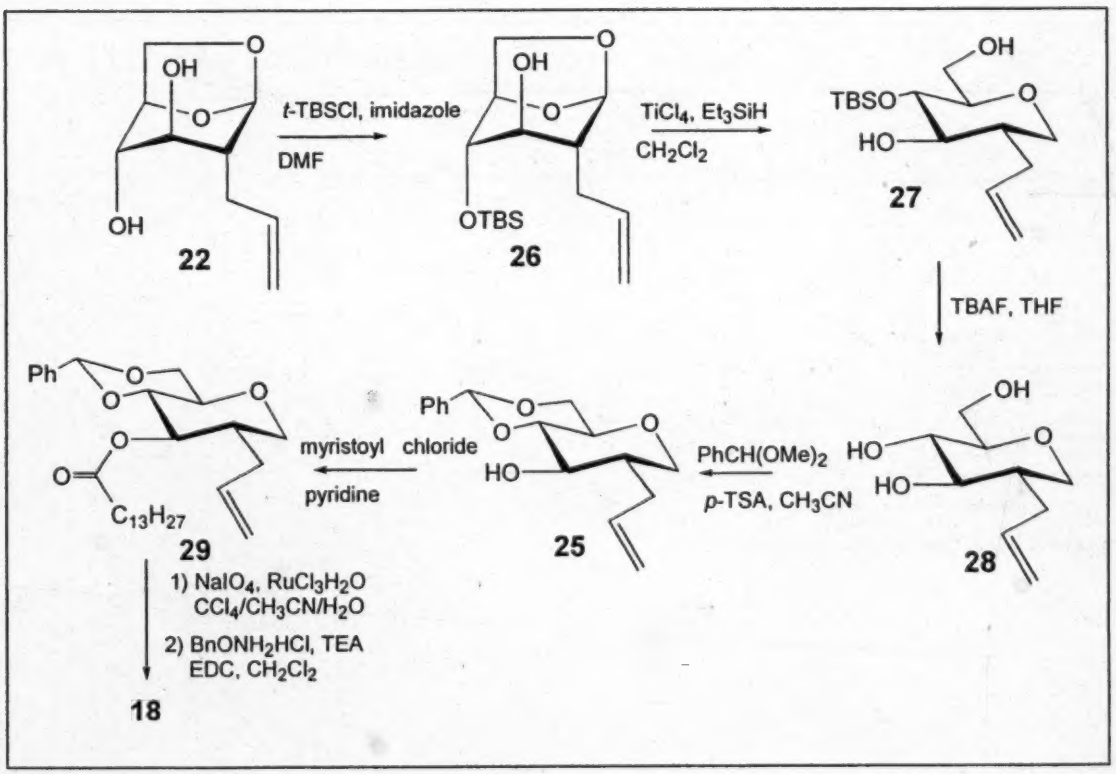
Initial attempts (Scheme 2.4) began with **21** or **22,** which were prepared according to the literature [42]. Reductive opening of the 1,6-anhydro ring of either compound was expected to afford the key intermediate **24**. However, this turned out not to be the case. On treatment of **21** with $SnCl_4$ and Et_3SiH, only starting material was observed on TLC even after two days, while the reaction of **22** gave multiple products in a short time.



Scheme 2.4

However, selective *tert*-butyldimethylsilyation of **22** gave **26** (81%) which, on treatment with $SnCl_4$ and Et_3SiH afforded **27** (67%) in 10 minutes. Use of $TiCl_4$ instead of $SnCl_4$ raised the yield of **27** to 80% but required 2 hours (Scheme 2.5). Desilyation of **27** with TBAF and subsequent acid-catalyzed reaction with benzaldehyde dimethylacetal gave **28** (85%), *O*-myristoylation of which gave **29** (98%). Oxidative cleavage of the double bond in **29** with ruthenium tetroxide, using the same conditions as in the first

pathway (Scheme 2.2), gave the carboxylic acid derivative where a hydroxamic acid function could be introduced near the end of the synthetic route, using *O*-benzylhydroxylamine/EDC to give the protected hydroxamic acid derivative **18** (70%). Finally, as in the first pathway, catalytic hydrogenation (H_2, Pd/C, AcOH) of **18** gave **1** (71%). This second synthetic pathway gave a 26.8% total yield of **1** (from **22**), as compared to 12.5% (from **13**) for the first pathway. Compound **29** was obtained in gram-scale. [44]



Scheme 2.5

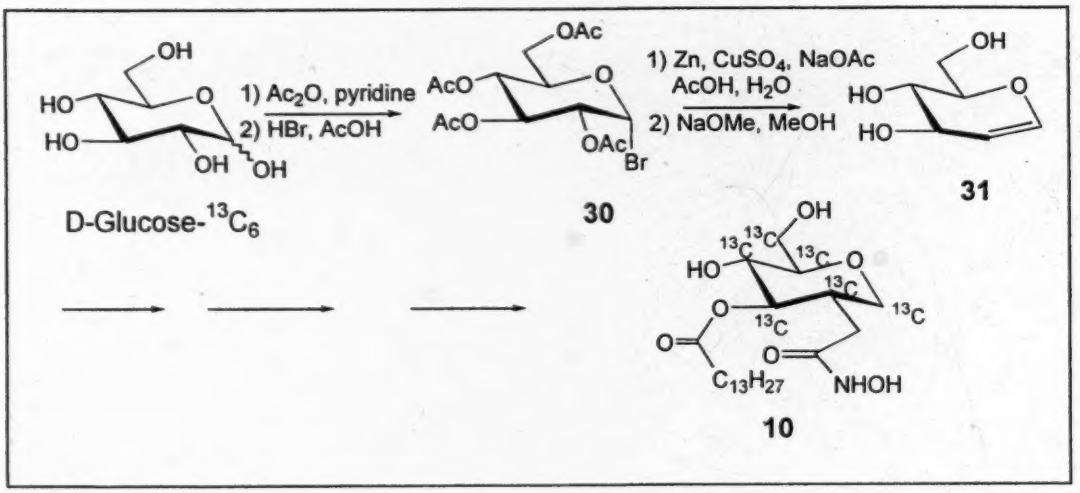
In summary, the 1,2-dideoxy-2-C-branched sugar was synthesized efficiently via reduction of 1,6-anhydro-β-D-glucopyranose with Et_3SiH/Lewis acid, but proper protecting groups were crucial for the transformation. Electron donating protecting groups, like benzyl and silyl ethers proved effective while the transformation was not successful with free hydroxyl or acetyl groups.

The solubility characteristics of **18** created difficulties in obtaining good NMR spectra. Its solubility in CD_3OD is very poor, and while its solubility in $CDCl_3$ is better, it provides very poor NMR spectra: some peaks are very broad, no splittings are observed in the ^1H NMR spectrum and some peaks are missing in the ^{13}C NMR spectrum. A mixture of CD_3OD and $CDCl_3$ therefore had to be used to produce high quality spectra.

Compound **1** was also resynthesized using uniformly ^{13}C labeled D-glucose as the starting material to provide ^{13}C labeled **1** (**10**) that would allow greater detail to be obtained on the solution structure of LpxC (*A. aeolicus*) in complex with inhibitor **1**. Compound **10** would help to increase the sensitivity of detection of intermolecular NOEs and make possible measurement of residual dipolar coupling on the sugar ring, eventually to obtain the conformation and relative orientation of the sugar-ring of **1** at high resolutions and with improved accuracy.

As shown in Scheme 2.6, beginning with commercial 99.9% uniformly ^{13}C labeled D-glucose, D-glucal-$^{13}C_6$ was obtained according to the literature [45]. The D-glucal was processed to the final compound **10** as described in Schemes 2.3, 2.4, and 2.5.

Compound **10** was obtained in 18 steps from D-Glucose-$^{13}C_6$ in an overall yield of 7% (50 mg) with a HRMS ($M+Na^+$) 460.2983 compared to compound **1** (454.2780).



Scheme 2.6

Figure 2.2 shows the ^{13}C NMR spectrum for **10** and the APT ^{13}C NMR spectrum for **1**. Additional splitting of ^{13}C resonance is observed for the ^{13}C-labeled molecule **10,** due to ^{13}C-^{13}C coupling.

Simplification of the ^1H NMR spectrum for ^{13}C labeled compound **10** (the first spectrum in Figure 2.3) was achieved via ^{13}C decoupling (the second one). As is evident in Figure 2.3, the ^{13}C-decoupled ^1H NMR spectrum of **10** bears close resemblance to the ^1H NMR spectrum of unlabeled compound **1** (the third one).

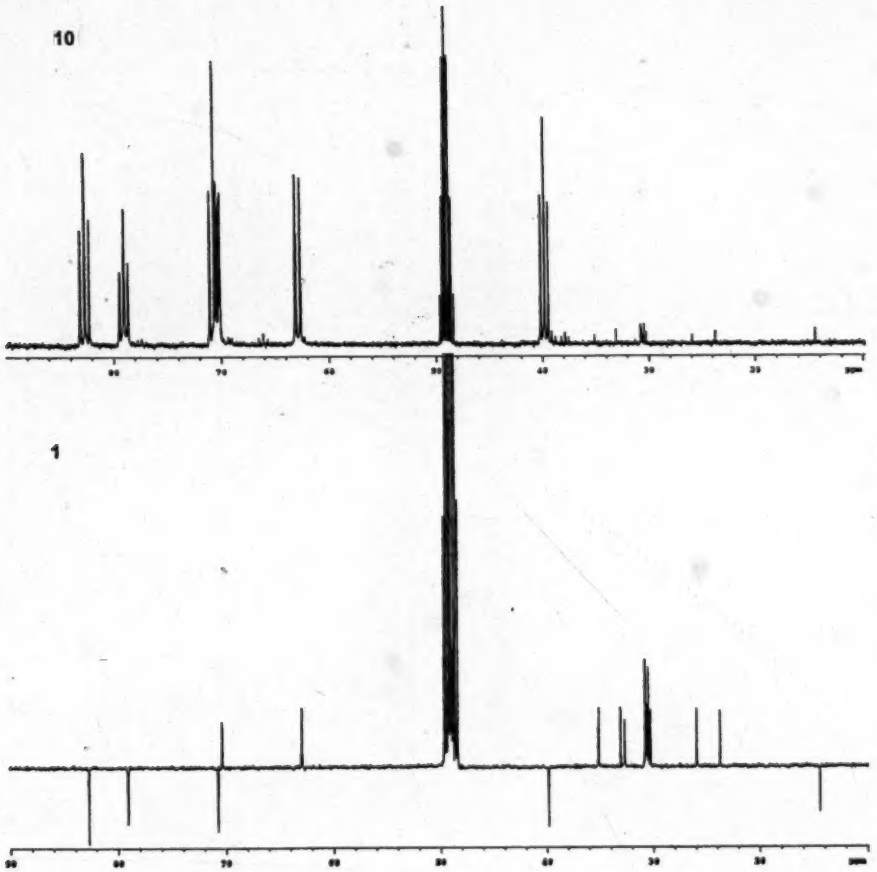


Figure 2.2. ^{13}C NMR spectra of **1** and **10** in CD_3OD

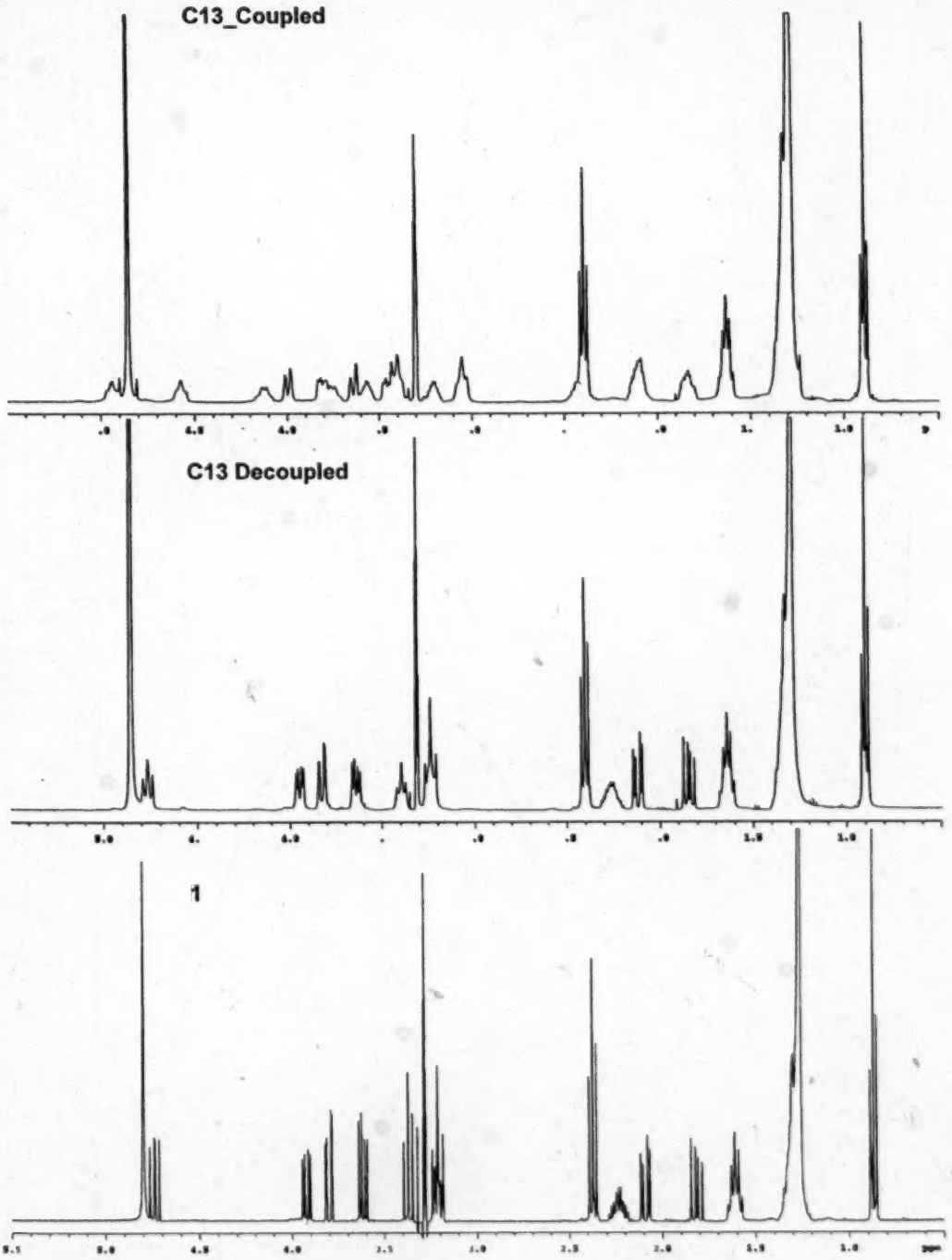
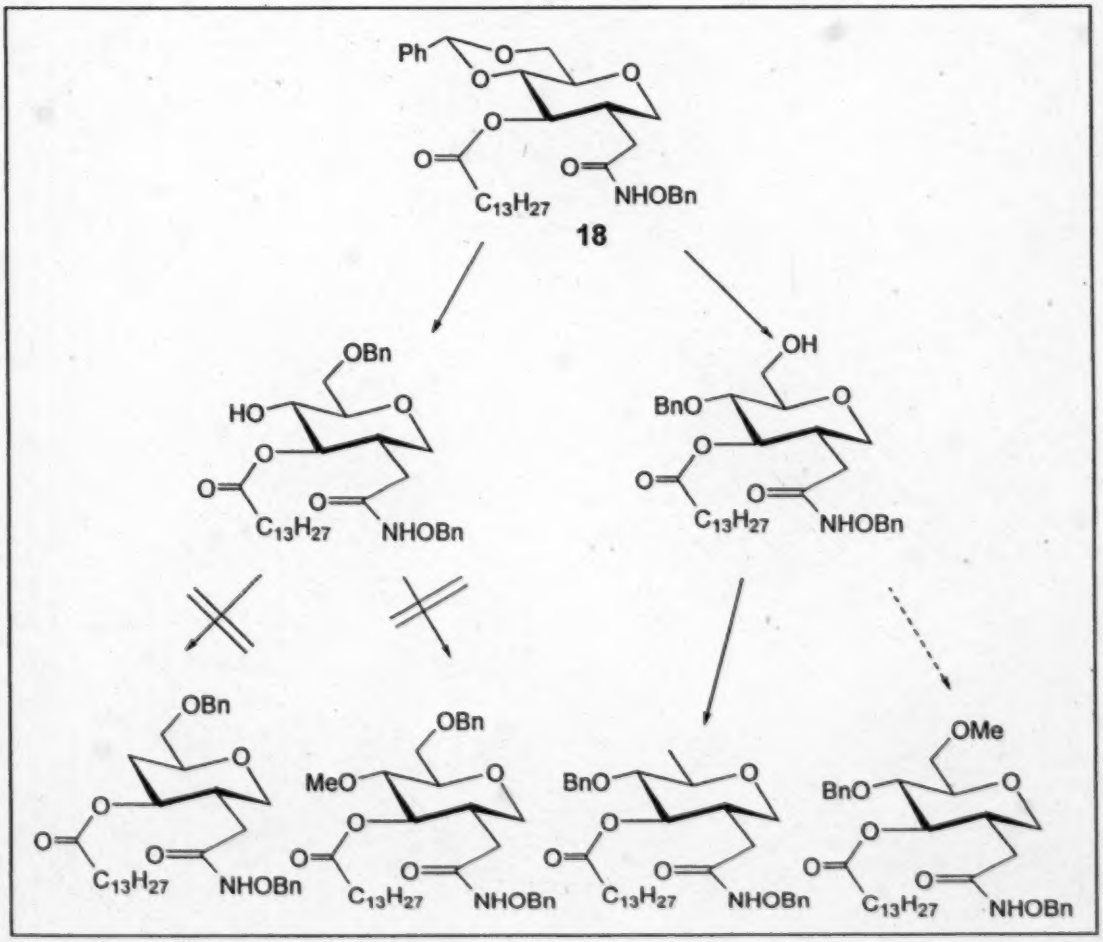


Figure 2.3. ^{1}H NMR spectra of **1** and **10** in CD_3OD

2.2.2 Preparation of modified single hydroxyl group analogs

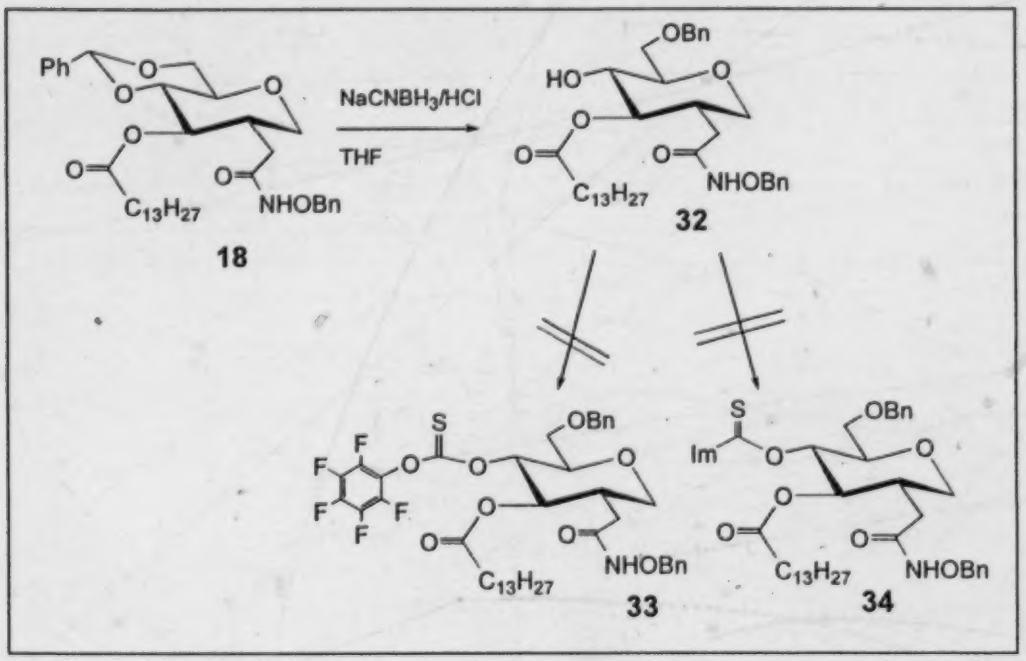
Our initial synthetic design was based on **18**, which is one of the intermediates in the first route to **1**. Compound **18** was expected to give the desired compounds rapidly (Scheme 2.7), but this route turned out to be very problematic as discussed below.



Scheme 2.7

Preparation of 1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,4-dideoxy-3-O-myristoyl-D-glucitol (2)

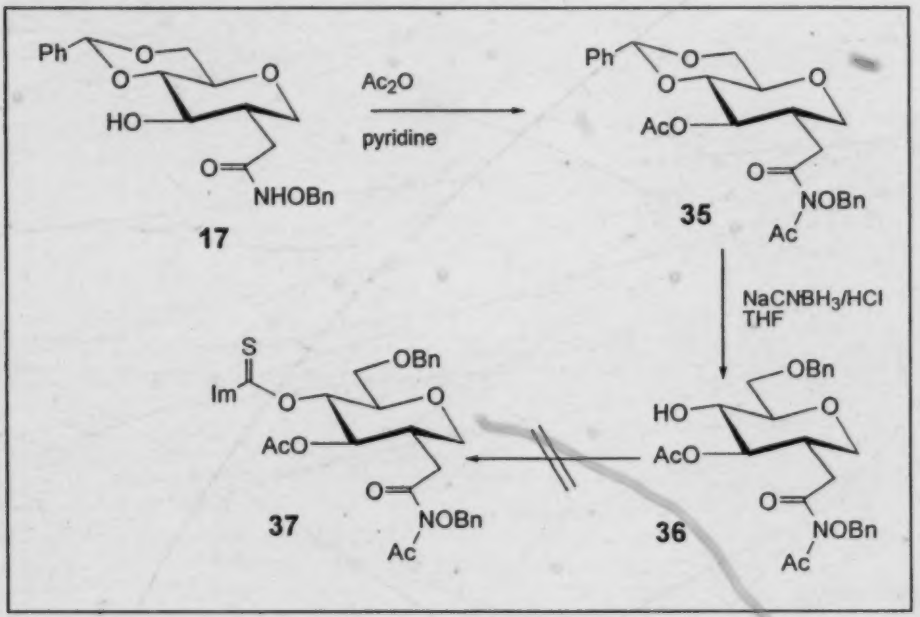
The first attempt to obtain the 4-deoxy analog **2** is shown in Scheme 2.8.



Scheme 2.8

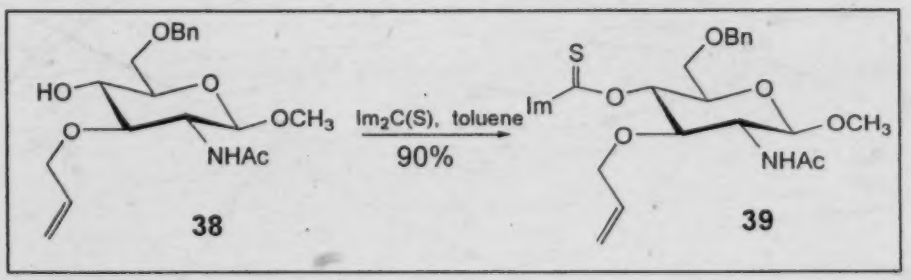
Selective reductive ring opening of benzylidene acetal in **18** using $NaCNBH_3$/HCl in THF gave the 6-O-benzyl ether **32** in 86% yield. However, attempted thiocarbonylation with either thiocarbonyldiimidazole or pentafluorophenyl chlorothionoformate under standard conditions failed to produce the desired products. It was assumed that the long chain at C-3 may have blocked the reaction, and that thiocarbonylation at the hydroxamic ester nitrogen might be also occurring. To circumvent this problem, we therefore *O*-acetylated **17** to produce **35** where the long

chain fatty acid ester was no longer present. At the same time, the hydroxamic ester nitrogen was also protected with acetyl group. Reductive ring opening of **35** now afforded **36** in good yield as expected (80%). Surprisingly, however, the attempted thiocarbonylation was still not successful.



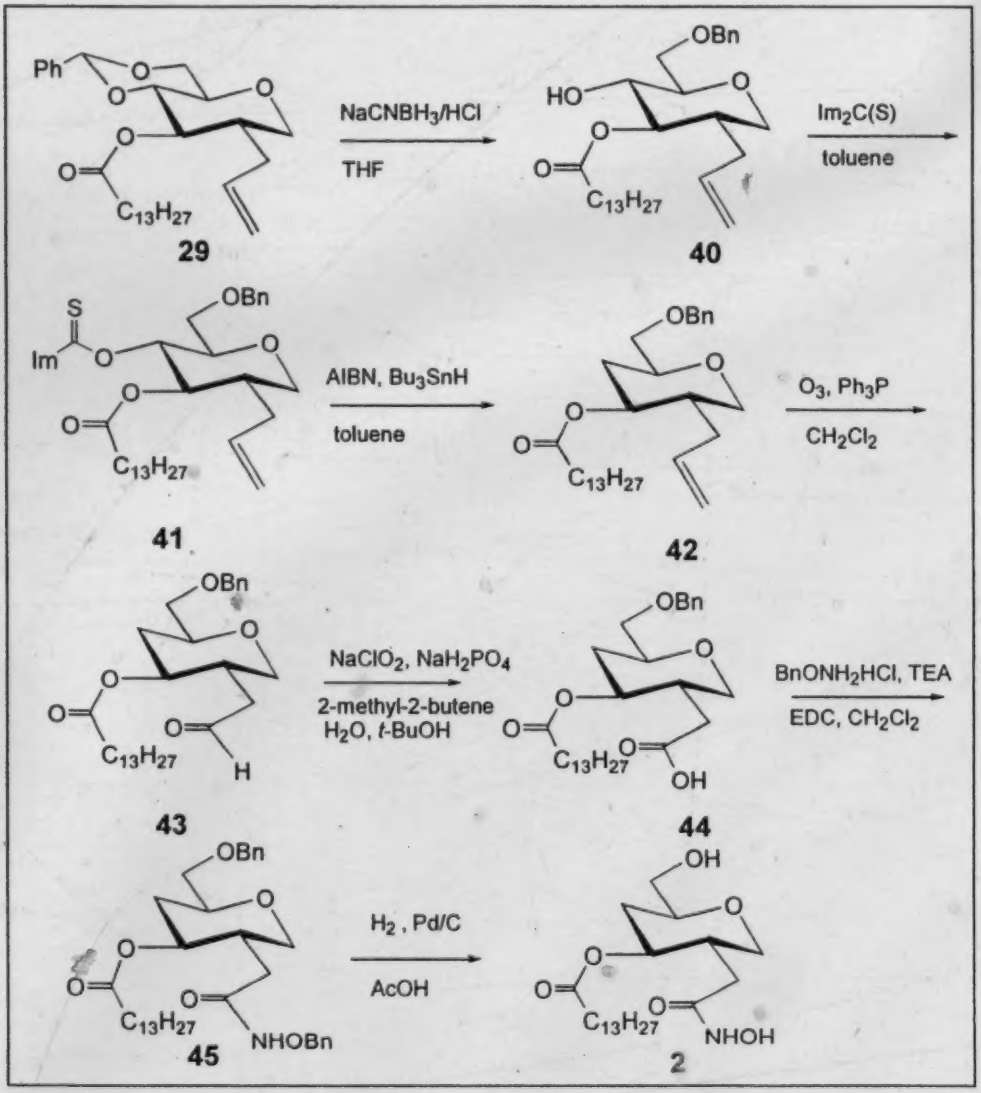
Scheme 2.9

We suspected that the problem might be with the reagents being used but under



Scheme 2.10.

the same reaction condition, compound **38** [46] gave **39** in 90% yield (Scheme 2.10). Therefore, we reasoned that the CONHOBn group may not be compatible with the thiocarbonylation conditions.



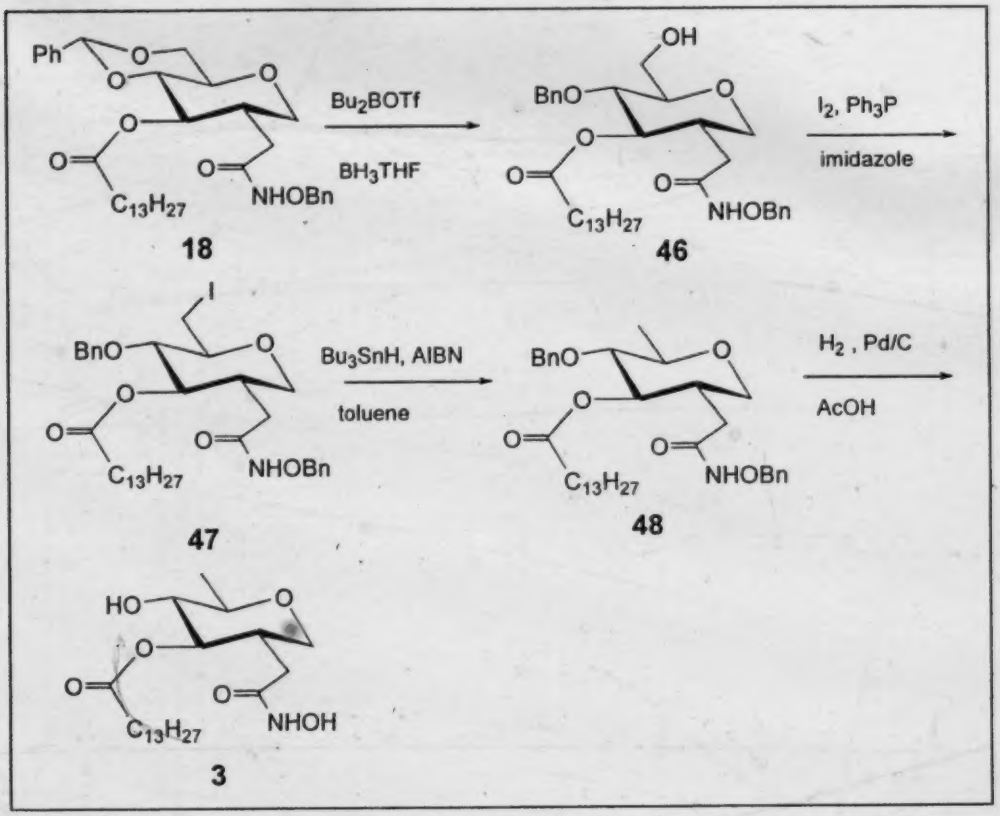
Scheme 2.11

Finally, the synthesis of the required 4–deoxy analog **2** was accomplished as shown in Scheme 2.11 after the second synthetic route to compound **1** was developed. Compound **29** is one of the intermediates in the second route (Scheme 2.5), which allowed us to remove the 4-OH group before the CONHOBn group was introduced. Regioselective reductive ring opening of the benzylidene acetal in **29** as above, followed by thiocarbonylation using thiocarbonyldiimidazole in boiling toluene overnight gave **41** in 64% over 2 steps. Treatment of **41** with Bu_3SnH and AIBN gave **42** in 85% yield. Ozonolysis of **42**, followed by reduction with PPh_3 gave aldehyde **43** (87%). Further oxidation to the carboxylic acid derivative using $NaClO_2$ and NaH_2PO_4 in *t*-BuOH/2-methyl-2-butene/H_2O yielded **44** (100%). This product was coupled with *O*-benzylhydroxylamine using EDC to afford the protected hydroxamic acid derivative **45** (70%). After hydrogenation (H_2, Pd/C) in AcOH, compound **2** was obtained (70%).

Preparation of 1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,6-dideoxy-3-O-myristoyl-D-glucitol (3)

Unlike the reductive benzylidene opening to yield the 6-O-benzyl ether, the conversion of the 4,6-O-benzylidene acetyl in **29** to the 4-benzyl ether proved very problematic. This is because the standard conditions used in this transformation, such as the combinations of $LiAlH_4/ACl_3$ or BH_3·THF/(Lewis acid), were not compatible with the presence of an ester group or a double bond. Selective reductive ring opening of the benzylidene acetal was finally achieved with Bu_2BOTf and BH_3·THF [47] from **18** (Scheme 2.12) to give **46** with OH-6 free (71%). Since the hydroxamic ester appeared

incompatible with the thiocarbonylation reagents, OH-6 was next replaced with iodine. Treatment of **46** with I_2, Ph_3P and imidazole afforded **47** in 80% yield. Deiodination with Bu_3SnH and AIBN successfully furnished **48** (82%). Hydrogenation (H_2, Pd/C, AcOH) of **48** then gave **3** (70%).



Scheme 2.12

The myristoyl ester in **3** was found to be labile towards migration which was observed when running a NMR spectrum in CD_3OD overnight to collect the required ^{13}C NMR data.

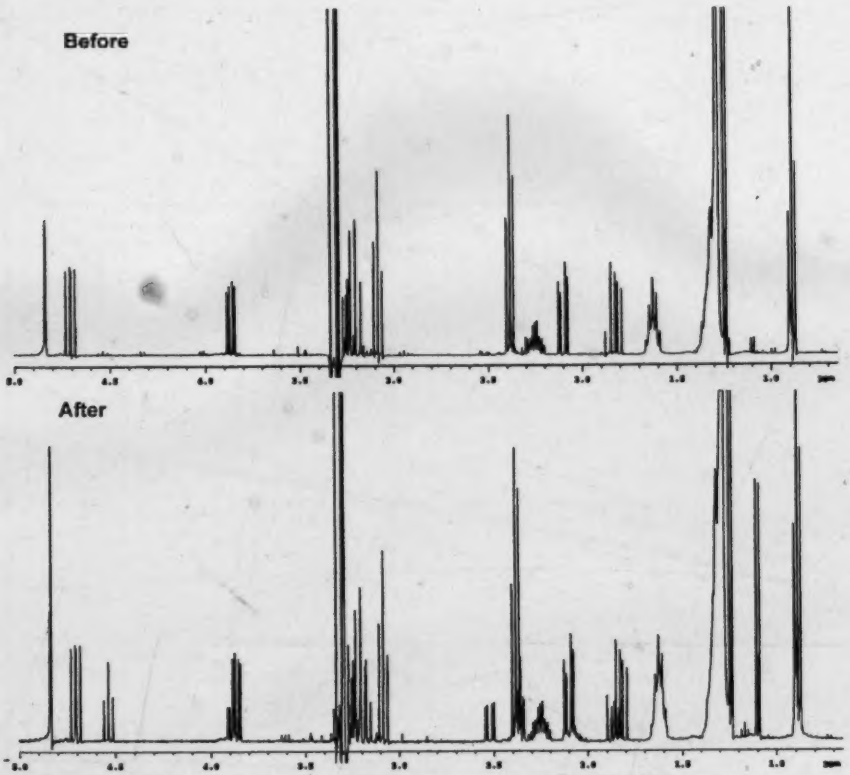
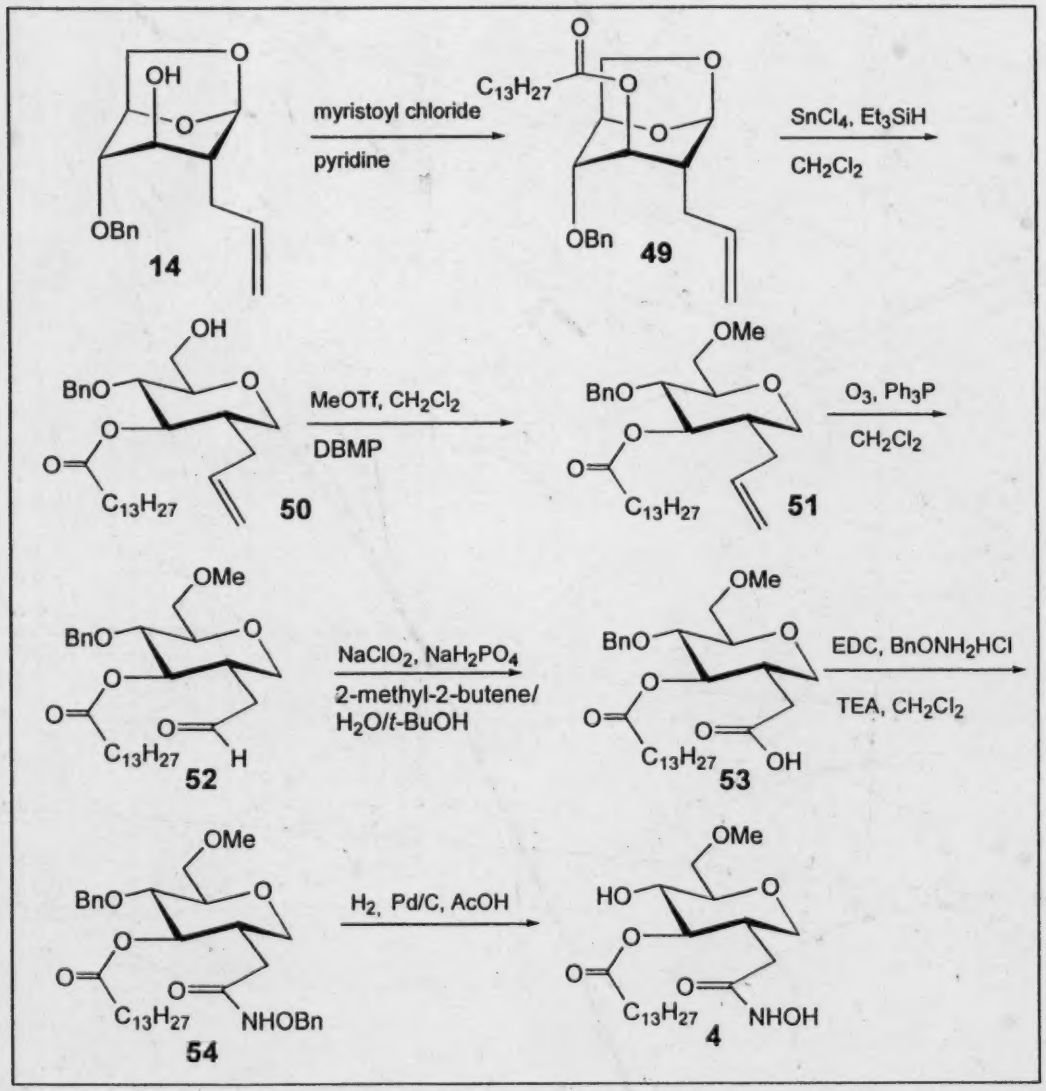


Figure 2.4. 1H NMR spectra of **3** before and after migration

Acyl migration was not evident in the earlier synthesis of **1**, so we reasoned that OH-4 would be less hindered in the 6-deoxy compound **3**, compared to **1**, which would facilitate the 3,4-myristoyl migration.

Preparation of 1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol (4)



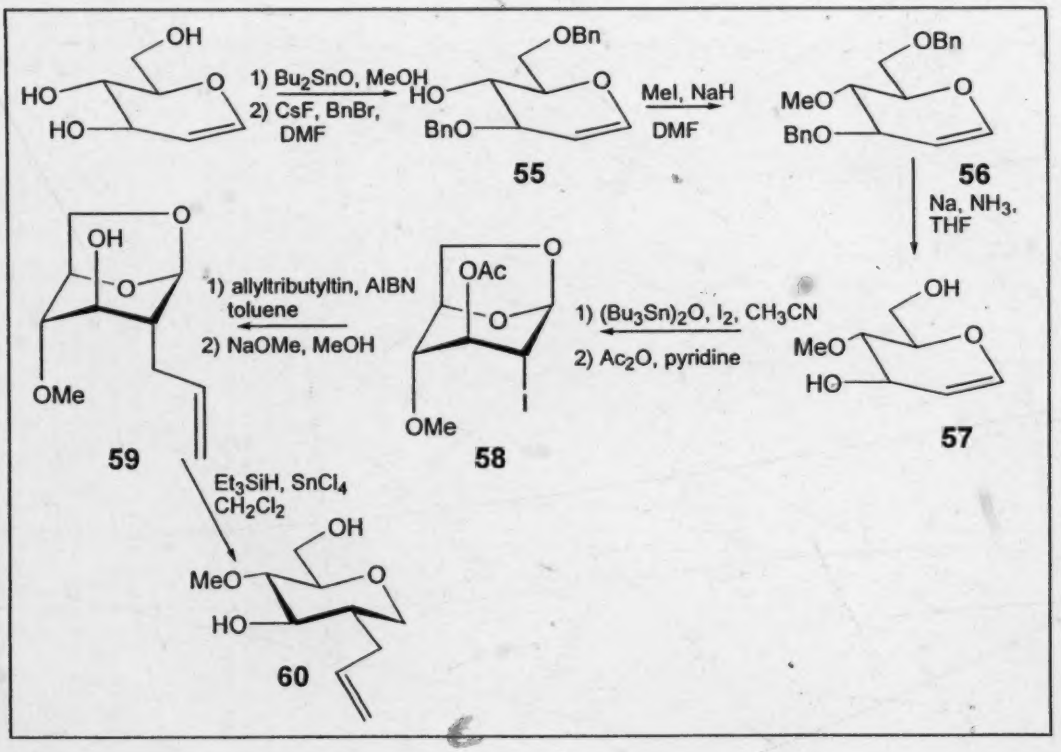
Scheme 2.13

The preparation of the 6-OMe derivative **4** would require an *O*-methylation reaction at some point. We therefore decided to install the methyl ether in the reaction

sequence before introduction of the CONHOBn group to avoid possible side reactions (Scheme 2.13). *O*-Myristoylation of **14** gave **49** (84%), which on treatment with $SnCl_4$ and Et_3SiH afforded **50** (46%). Methylation of **50** was easily accomplished. Treatment with methyl trifluoromethanesulfonate and 2,6-di(tert-butyl-4-methyl) pyridine gave **51** (98%). Finally as in Scheme 2.11, the reaction sequence was completed by ozonolysis followed by reduction to the aldehyde **52** (80%), and further oxidation to carboxylic acid **53** (100%). Coupling with O-benzylhydroxylamine hydrochloride ($BnONH_2 \cdot HCl$) gave the hydroxamide **54** (71%), and upon hydrogenation (H_2, Pd/C, AcOH), compound **4** was obtained (69%).

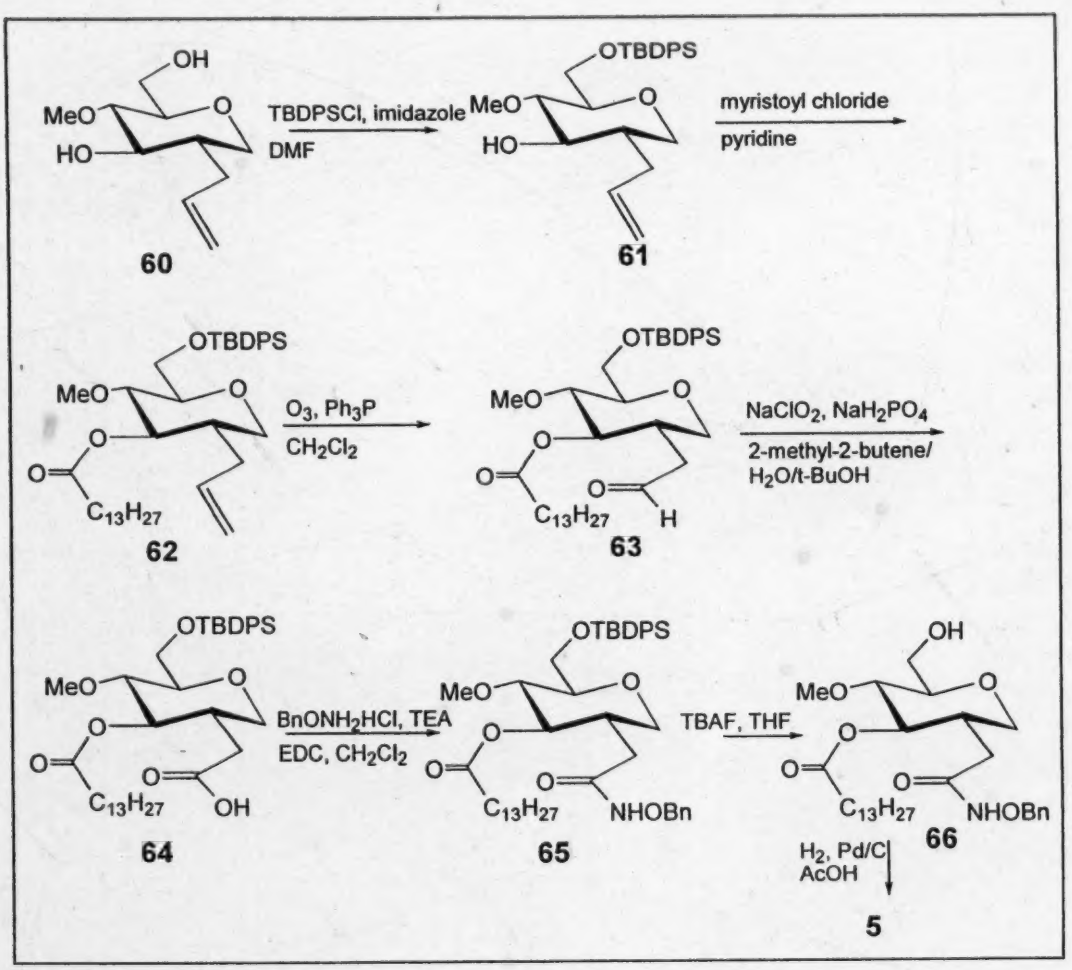
Preparation of 1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-4-O-methyl-3-O-myristoyl-D-glucitol (5)

The preparation of 4-*O*-methyl derivative **5** is shown in Scheme 2.14 and Scheme 2.15. Compound **56**, obtained from D-glucal according to the literature [48], was subjected to Birch reduction to afford **57** (85%). Cyclization of **57** gave **58** (89%) using the same reaction conditions with Scheme 2.3. Radical addition of allyltributylstannane with AIBN as the initiator, followed by deacetylation gave **59** in 69% yield. Reductive opening of the 1,6-anhydro ring with $SnCl_4$ and Et_3SiH gave **60** (66%).



Scheme 2.14

Selective *t*-butyldiphenylsilyation at the primary alcohol of **60** gave **61** (97%) (Scheme 2.15). *O*-Acylation with myristoyl chloride in pyridine then gave **62** (95%). Ozonolysis of **62**, followed by reduction using PPh_3 gave aldehyde **63** (81%). Further oxidation using $NaClO_2$ and NaH_2PO_4 in *t*-BuOH/2-methyl-2-butene/H_2O yielded **64** quantitatively. This carboxylic acid coupled with *O*-benzylhydroxylamine using EDC to furnish **65** (68%). The *t*-butyldiphenylsilyl group was removed by TBAF to give **66** (91%). Hydrogenation of **66** gave **5** (64%).

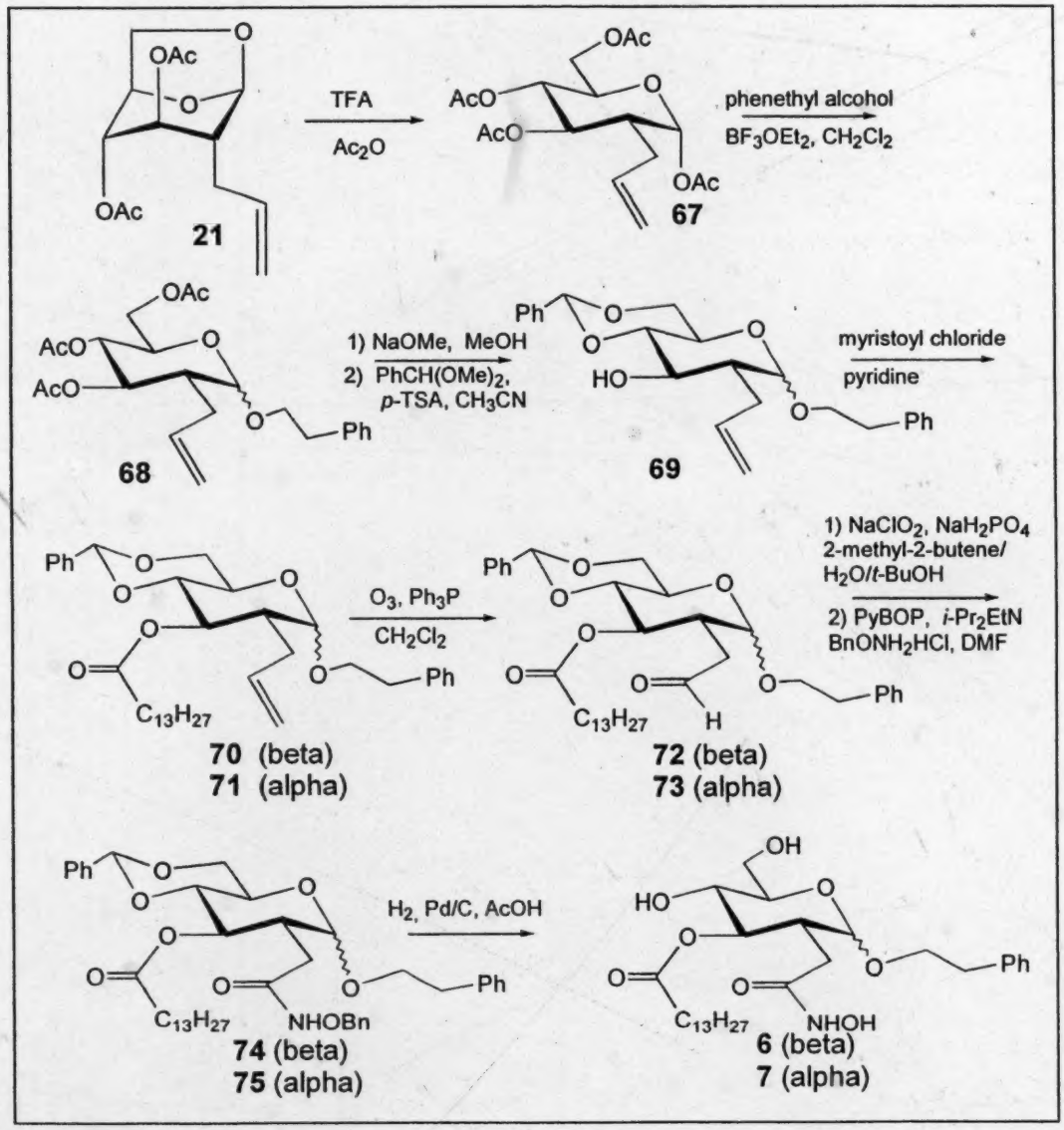


Scheme 2.15

2.2.3 Preparation of C_1-modified analogs (6 and 7)

As shown in Scheme 2.16, trifluoroacetolysis of the 1,6-anhydro linkage of **21** gave rise to the α acetate **67** (100%), which was coupled with phenethyl alcohol under $BF_3{\cdot}OEt_2$ catalysis, yielding **68** (95%). The 1:1 mixture of anomers was inseparable by silica gel chromatography. Deacylation of **68** and subsequent acid-catalyzed reaction with

benzaldehyde dimethylacetal gave an inseparable anomeric mixture **69** (94%). O-Acylation of **69** with myristoyl chloride then gave **70** (42%) and **71** (45%). At this stage, the anomeric mixture could finally be separated by silica gel chromatography.



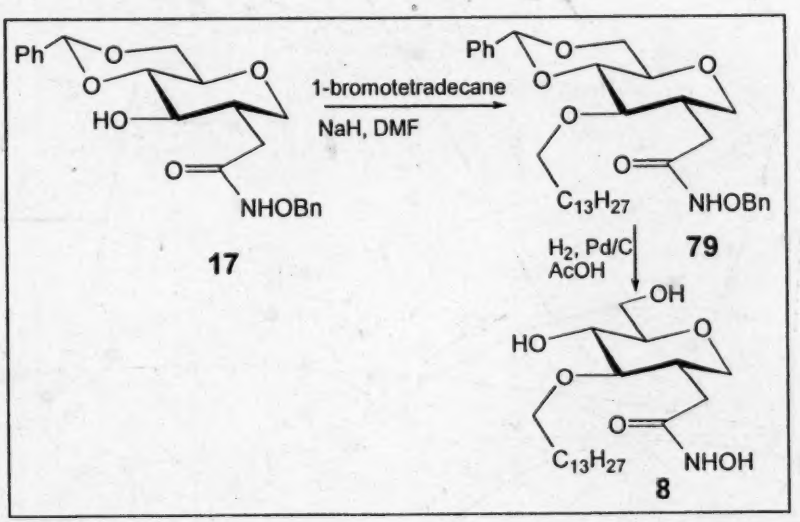
Scheme 2.16

Respectively, ozonolysis of **70** and **71**, followed by reduction with PPh_3 gave aldehyde **72** (80%) and **73** (93%). Further oxidation using $NaClO_2$ and NaH_2PO_4 in MeCN/*t*-BuOH/2-methyl-2-butene/H_2O afforded carboxylic acid derivatives quantitatively. The products were coupled with *O*-benzylhydroxylamine after activating the acid with PyBOP to afford the protected hydroxamic acid derivatives **74** (80%) and **75** (82%). Hydrogenation of **74** and **75** (H_2, Pd/C) in AcOH yielded compounds **6** (60%) and **7** (62%).

2.2.3 Preparation of OH-3 modified analogs (8, 9a and 9b)

Compounds **9a** and **9b** were prepared according to the same reaction sequence with **1**, using benzoyl chloride and palmitoyl chloride instead of myristoyl chloride.

The preparation of **8** is shown in Scheme 2.17.

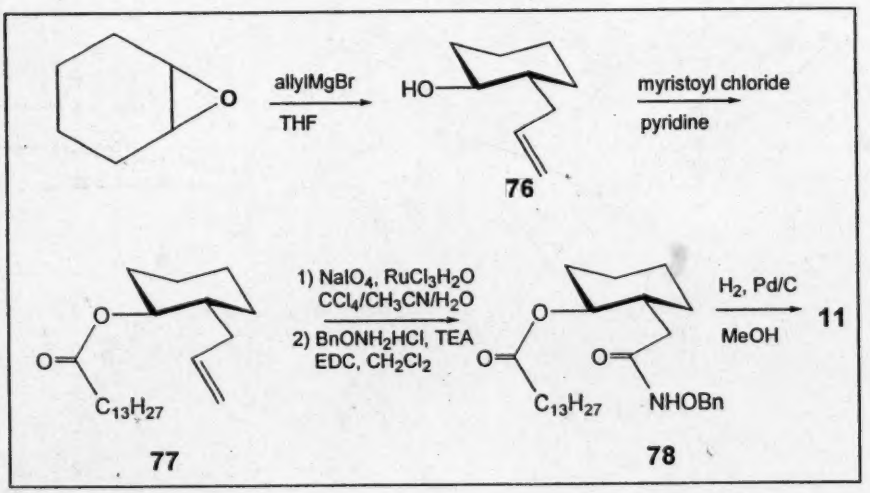


Scheme 2.17

Fortunately, treatment of **17** with NaH and 1-bromotetradecane in DMF, quenched before the reaction was completed, gave **79** (70%). *O*-Alkylation instead of *N*-alkylation was confirmed by the presence of a NH peak in the ^{1}H NMR spectrum. After hydrogenation, compound **8** was obtained (70%).

2.2.4 Synthesis of control compounds (11 and 83)

The preparation of tetradecanoic acid 2-(carboxymethyl N-hydroxyamide)-cyclohexyl ester (**11**) is shown in Scheme 2.18.



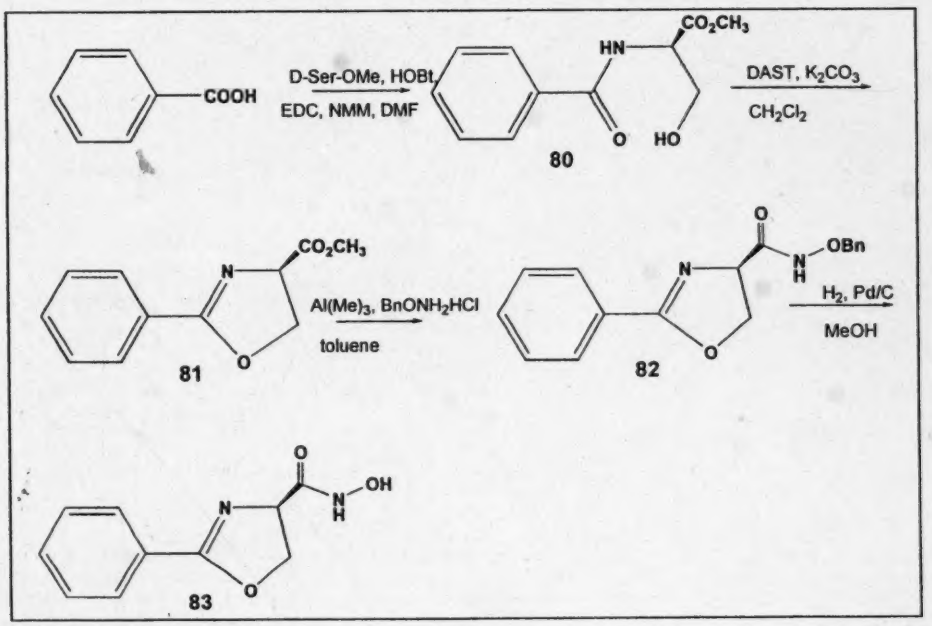
Scheme 2.18

Treatment of cyclohexene oxide with allylmagnesium bromide afforded **76** (racemic) according to literature [49]. *O*-Acylation with myristoyl chloride then gave **77** (80%). Oxidation of the double bond in **77** with ruthenium tetroxide (generated in situ from $RuCl_3$ and $NaIO_4$) gave a carboxylic acid derivative. The crude product was coupled

with *O*-benzylhydroxylamine using EDC to give the protected hydroxamic acid derivative **78** (66%). Hydrogenation of **78** afforded **11** (racemic) (75%).

Merck inhibitor L-159, 463 (83)

The compound L-159, 463 (**83**) [50] was prepared according to the literature as a reference compound (Scheme 2.19). Benzoic acid was coupled with D-serine methyl ester to afford **80** which was cyclized to the oxazolidine derivative **81** by DAST and K_2CO_3. The oxazolidine methyl ester was reacted with the Weinreb reagent to give **82** and then the benzyl group was removed by hydrogenation to give the **83**.



Scheme 2.19

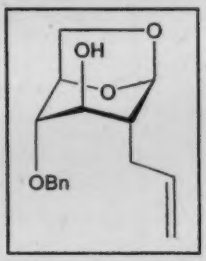
2.3 Experimental

2.3.1 General Methods

Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in EtOH. All commercial reagents were used as supplied. Column chromatography was performed on Silica Gel 60 (E. Merck 40 – 60 μM, Darmstadt). Iatrobeads (beaded silica gel 6RS-8060) were from Iatron Labotatories (Tokyo). ^{1}H NMR spectra were recorded on 300 MHz (Varian Inova 300), 400 MHz (Varian Inova 400), 500 MHz (Varian Inova 400) or 600 MHz (Varian Inova 600). The first order proton chemical shifts δ_H are referenced to either residual $CHCl_3$ (δ_H 7.24, $CDCl_3$) or CH_3OD (δ_H 3.30, CD_3OD). HMQC NMR spectra were recorded on Varian Unity 300 MHz, 400 MHz, 500 MHz or 600 MHz NMR spectrometers. The ^{13}C chemical shifts, δ_C are referenced to internal $CDCl_3$ (δ_C 77.00), CD_3OD (δ_C 49.00). The diastereotopic methylene protons of benzyl groups are reported as ABd, meaning second order doublets, with the chemical shifts reported as the mid-point between the peaks. Organic solutions were dried prior to concentration under vacuum at < 40 °C (bath). Microanalyses and electrospray mass spectra were performed by the analytical services of this department.

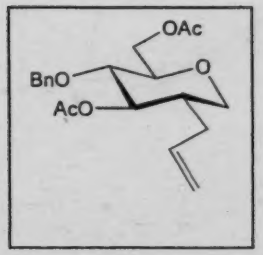
2.3.2 Chemical synthesis

1, 6-Anhydro-4-O-benzyl-2-deoxy-2-C-(2-propenyl)-β-D-glucopyranose **(14)**



To a solution of "Cerny's epoxide" **13** (1.01 g, 4.30 mmol) in THF (10 ml), allylmagnesium bromide (1.0 M in Et_2O, 12.8 ml, 12.8 mmol) was added slowly at room temperature and the reaction mixture was gently refluxed at 60 °C for 2 hours. After cooling to room temperature, sat. NH_4Cl (10 ml) was slowly added and the mixture was extracted with EtOAc (30 ml x 3). The combined organic layer was washed with brine (10 ml x 2), dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene /EtOAc, 8:1) to afford **14** (931 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (5H, m), 5.79 (1H, m, H-2'), 5.45 (1H, br s, H-1), 5.12-5.05 (2H, m, H-3'), 4.66 and 4.60 (2H, ABd, J = 12.0 Hz, OCH_2Ph), 4.58 (1H, m, H-5), 4.05 (1H, dd, J = 0.7, 7.5 Hz, H-6), 3.70-3.64 (2H, m, H-3 and H-6), 3.40 (1H, s, H-4), 2.30 (2H, m, H-1'), 1.79 (1H, br t, J = 7.0 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 135.8, 128.4, 127.8, 127.6, 117.2, 103.4, 78.9, 74.9, 71.4, 69.1, 65.5, 45.7, 33.5; HRMS calcd for $C_{16}H_{20}O_4Na$ ($M+Na^+$) 299.1259, found 299.1259

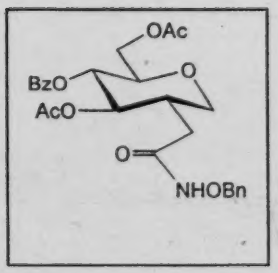
3, 6-Di-O-acetyl-1, 5-anhydro-4-O-benzyl-2-deoxy-2-C-(2-propenyl)-D-glucitol **(15)**



A solution of **14** (760 mg, 2.75 mmol) in dry CH_2Cl_2 (50 ml) was cooled to 0 °C under argon. Et_3SiH (2.20 ml, 13.8 mmol) was

added followed by $SnCl_4$ (0.49 ml, 4.13 mmol) at 0 °C and the mixture was stirred for 1 hour at room temperature. The reaction mixture was poured into cold sat. $NaHCO_3$ (20 ml) and the aqueous layer was extracted with CH_2Cl_2 (20 ml x 3) at once. The combined organic layer was washed with brine (5 ml), dried over Na_2SO_4 and concentrated. The residue was directly acetylated using Ac_2O (4 ml) and pyridine (5 ml) for overnight. After removing the solvent, the residue was purified by chromatography (toluene/EtOAc, 9:1) to afford **15** (877 mg, 88%); ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (5H, m), 5.64 (1H, m, H-2'), 4.95 (3H, m, H-3' and H-3), 4.54 and 4.48 (2H, ABd, J = 11.1 Hz, OCH_2Ph), 4.28 (1H, d, J = 11.9 Hz, H-6), 4.16 (1H, dd, J = 4.1, 11.9 Hz, H-6), 3.88 (1H, dd, J = 4.5, 11.5 Hz, H-1eq), 3.40 (2H, m, H-4 and H-5), 3.30 (1H, t, J = 11.5 Hz H-1ax), 2.10 (1H, m, H-1'), 2.00 (3H, s, OAc), 1.94 (3H, s, OAc), 1.88-1.80(2H, m, H-1' and H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 137.6, 134.8, 128.4, 127.8, 127.8, 116.9, 77.7, 77.6, 76.9, 74.5, 69.8, 63.4, 41.0, 32.2, 20.9, 20.8; HRMS calcd for $C_{20}H_{26}O_6Na$ ($M+Na^+$) 385.1622, found 385.1613

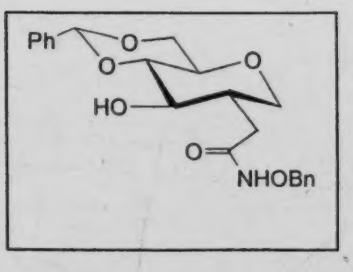
3, 6-Di-O-acetyl-1, 5-anhydro-4-O-benzoyl-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-D-glucitol **(16)**



To a solution of **15** (150 mg, 0.41 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:1, 15 ml), $NaIO_4$ (443 mg, 0.41 mmol) and $RuCl_3 \cdot H_2O$ (10 mg, 0.05 mmol) were successively added at room temperature. The reaction mixture was stirred vigorously for 2 hours and then extracted with CH_2Cl_2 (15 ml x 4). The combined organic layers

were dried over Na_2SO_4 and concentrated to afford crude acid (140 mg, 86%). This crude product was used directly in the next step. To a solution of the crude product and $BnONH_2 \cdot HCl$ (57 mg, 0.36 mmol) in CH_2Cl_2 (4 ml), EDC (82 mg, 0.43 mmol) and triethylamine (60 µl, 0.43 mmol) were successively added at room temperature and stirred for 3 hours. CH_2Cl_2 (15 ml) was added and the solution was washed with 1N HCl (5 ml), H_2O (5 ml) and brine (5 ml). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 3:2) to afford **16** (157 mg, 76%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.99-7.25 (10H, m), 5.12 (2H, m, H-3 and H-4), 4.84 and 4.80 (2H, ABd, J = 10.9 Hz, OCH_2Ph), 4.15 (1H, dd, *J* = 4.9, 12.2 Hz, H-6), 4.08 (1H, dd, *J* = 2.8, 12.2 Hz, H-6), 3.96 (1H, dd, *J* = 4.8, 11.5 Hz, H-1eq), 3.74 (1H, m, H-5), 3.14 (1H, t, *J* = 11.5 Hz, H-1ax), 2.40 (1H, m, H-2), 2.18 (1H, dd, *J* = 5.0, 14.8 Hz, H-1'), 1.98-1.96 (4H, s, OAc and H-1'), 1.82 (3H, s, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171. 1, 170.7, 167.8, 165.4, 135.3, 133.5, 129.3, 128.8, 125.2, 78.1, 76.5, 74.9, 70.5, 69.4, 63.1, 38.0, 31.4, 21.4, 20.6, 20.6; HRMS calcd for $C_{26}H_{29}NO_9Na$ ($M+Na^+$) 522.1740, found 522.1745

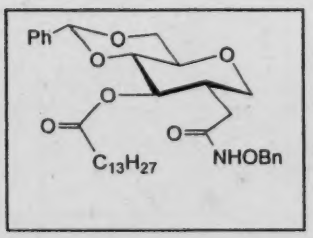
1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-D-glucitol **(17).**



To a solution of **16** (120 mg, 0.24 mmol) in MeOH (5 ml), NaOMe (catalytic) was added at room temperature and stirred for 3 hours. The reaction mixture was then neutralized using Amberlite IR-120 (H^+), filtered and

concentrated. The solid residue was directly used in the next step. To a suspension of the crude product in CH_3CN (30 ml), benzaldehyde dimethylacetal (54 µl, 0.36 mmol) and $TsOH{\cdot}H_2O$ (7 mg, 0.04 mmol) were successively added at room temperature. The suspension could become clear within 15 minutes. After 3 hours, triethylamine (100 µl) was added to the reaction mixture at 0 °C and the solvent was removed by evaporation. The residue was purified by chromatography (toluene/EtOAc, 1:1) to give **17** (61 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD = 2:1) δ 7.38-7.10 (10H, m), 5.40 (1H, s, CHPh), 4.68 (2H, overlapped with H_2O, OCH_2Ph), 4.08 (1H, dd, J = 4.9, 10.3 Hz, H-6), 3.78 (1H, dd, J = 4.8, 11.5 Hz, H-1eq), 3.59 (1H, t, J = 10.3 Hz, H-6), 3.28 (2H, m, H-3 and H-4), 3.16 (1H, m, H-5), 3.10 (1H, t, J =11.5 Hz, H-1ax), 2.28 (1H, dd, J = 4.5, 12.7 Hz, H-1'), 1.98 (1H, m, H-2), 1.72 (1H, dd, J = 8.2, 12.7 Hz, H-1'); ^{13}C NMR (125 MHz, $CDCl_3$: CD_3OD = 2:1) δ 169.3, 137.0, 135.1, 128.9, 128. 8, 128.4, 128.2, 125.9, 101.7, 82.9, 77.8, 72.6, 71.5, 70.1, 68.6, 40.4, 31.6; HRMS calcd for $C_{22}H_{25}NO_6Na$ ($M+Na^+$) 422.1580, found 422.1581

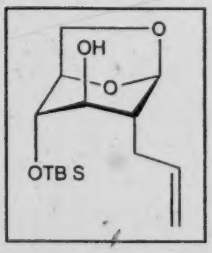
1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol **(18).**



To a solution of **17** (20 mg, 50 µmol) in pyridine (0.5 ml), DMAP (3 mg, 30 µmol) and myristoyl chloride (27 µl, 0.10 mmol) were successively added at 0 °C and stirred for 18 hours at room temperature. EtOAc (30 ml) was then added to the reaction mixture and the solution was washed with 10% citric acid (5 ml x 3), H_2O (5

ml), sat. $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 4:1) to afford **18** (16 mg, 52%). ^1H NMR (400 MHz, CD_3OD: $CDCl_3$ = 2:1) δ 7.40-7.24 (10H, m), 5.58 (1H, s, CHPh), 5.01 (1H, dd, *J* = 9.4, 10.6 Hz, H-3), 4.82 (2H, overlapped with H_2O, OCH_2Ph), 4.21 (1H, dd, *J* = 4.9, 10.4 Hz, H-6), 3.92 (1H, dd, *J* = 4.9, 11.5 Hz, H-1eq), 3.72 (1H, t, *J* = 10.4 Hz, H-6), 3.62 (1H, t, *J* = 9.4 Hz, H-4), 3.41 (1H, m, H-5), 3.32 (1H, t, *J* = 11.5 Hz, H-1ax), 2.40 (1H, m, H-2), 2.33 (2H, t, *J* = 7.3 Hz, $OC(O)CH_2$), 2.14 (1H, dd, *J* = 4.8, 14.7 Hz, H-1'), 1.88 (1H, dd, *J* = 14.7, 9.0 Hz, H-1'), 1.56 (2H, m), 1.24 (20H, m), 0.89 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD: $CDCl_3$ = 2:1) δ 175.3, 170.1, 138.9, 136.9, 130.4, 129.9, 129.8, 129.6, 129.1, 127.3, 102.7, 81.9, 74.8, 73.1, 71.1, 69.8, 39.9, 35.3, 33.1, 32.5, 30.8, 30.8, 30.6, 30.5, 30.1, 26.2, 23.8, 14.6; HRMS calcd for $C_{36}H_{51}NO_7Na$ ($M+Na^+$) 632.3563, found 632.3568

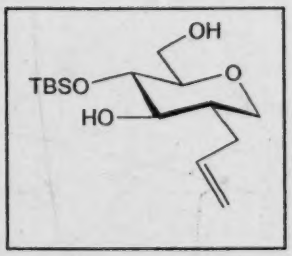
1, 6-Anhydro-4-O-(tert-butyldimethyl) silyl-2-deoxy-2-C-(2-propenyl)-β-D-glucopyranose **(26).**



To a solution of **22** (670 mg, 3.60 mmol) and imidazole (980 mg, 14.4 mmol) at 0 °C in DMF (12 ml) was added *tert*-butyldimethylsiyl chloride (651 mg, 4.34 mmol). The solution was allowed to slowly warm to room temperature overnight (11 hours). Additional *tert*-butyldimethylsily chloride (100 mg) was then added. After being stirred for an additional 1 hour, the solution was poured into an Erlenmeyer flask, diluted with 25 ml saturated $NaHCO_3$, 25 ml water, and 50 ml Et_2O and then stirred vigorously for 15 min. The layers

were separated and the aqueous layer was extracted with Et_2O (30 ml x 3). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, filtered, concentrated and purified by chromatography (EtOAc/hexane, 1:10) to yield **26** (875 mg, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.80 (1H, m, H-2'), 5.45 (1H, br s, H-1), 5.13-5.05 (2H, m, H-3'), 4.36 (1H, d, J = 5.3 Hz, H-5), 4.12 (1H, dd, J = 0.5, 7.5 Hz, H-6), 3.70-3.65 (2H, m, H-6 and H-4), 3.51 (1H, m, H-3), 2.30 (2H, m, H-1'), 1.78 (1H, br t, J = 7.0 Hz, H-2), 0.92 (9H, s), 0.10 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.0, 116. 8, 103.5, 77.4, 73.0, 72.3, 65.2, 45.8, 33.4, 25.7, 18.0, -4.9, -5.0; HRMS calcd for $C_{15}H_{28}O_4SiNa$ ($M+Na^+$) 323.1655, found 323.1653

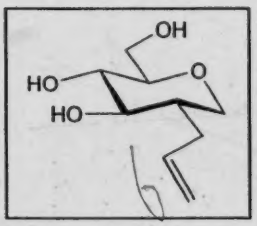
1, 5-Anhydro-4-O-(tert-butyldimethyl) silyl-2-deoxy-2-C-(2-propenyl)-D-glucitol **(27).**



To a solution of **26** (1.00 g, 3.33 mmol) and Et_3SiH (2.10 ml, 13.2 mmol) in CH_2Cl_2 (50 ml), $TiCl_4$ (3.95 ml, 1.0 M in CH_2Cl_2, 3.95 mmol) was added dropwise at – 78 °C, allowing the reaction to warm to – 15 °C over 2 hours. The reaction was poured into cold sat. $NaHCO_3$ (20 ml) and extracted with CH_2Cl_2 (20 ml x 2). The combined organic layers were washed with brine (10 ml), and dried with Na_2SO_4. The residue was purified by chromatography (EtOAc/hexane, 1:7) to afford **27** (800 mg, 80%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 (1H, m, H-2'), 5.09-4.99 (2H, m, H-3'), 3.91 (1H, dd, J = 4.8, 11.7 Hz, H-1eq), 3.82 (1H, dd, J = 2.7, 11.7 Hz, H-6), 3.63 (1H, dd, J = 5.6, 11.7 Hz, H-6), 3.49 (1H, t, J = 9.0 Hz, H-4), 3.27 (1H, J = 9.0 Hz, H-3), 3.17 (2H, m, H-5 and H-1 ax), 2.54 (1H, m, H-1'), 1.96 (1H, m, H-2), 1.76 (1H, m, H-1'), 0.90 (9H, s),

0.06 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.7, 116.7, 80.6, 77.6, 73.3, 69.9, 62.3, 41.9, 32.6, 25.9, 18.2, -3.9, - 4.8; HRMS calcd for $C_{15}H_{30}O_4SiNa$ ($M+Na^+$) 325.1811, found 325.1816

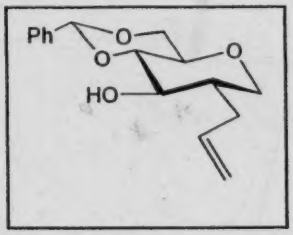
1, 5-Anhydro-2-deoxy-2-C-(2-propenyl)-D-glucitol **(28).**



A solution of **27** (1.00 g, 3.31 mmol) at room temperature in THF (40 ml) was treated with TBAF (1.0 M solution in THF, 8.50 ml, 8.50 mmol) under argon. After 2.5 hours, the reaction was concentrated and purified directly by chromatography (CH_2Cl_2 /MeOH, 15:1).

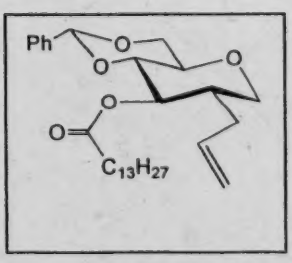
Note: it was difficult to remove the residue of TBAF from the product, so it was acetylated with Ac_2O and pyridine and purified by chromatography (EtOAc/hexane, 1:2). Deacetylation with NaOMe in MeOH then gave pure **28** (585 mg, 94%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.80 (1H, m, H-2'), 5.05-4.95 (2H, m, H-3'), 3.87 (1H, dd, J = 4.7, 11.7 Hz, H-1eq), 3.80 (1H, dd, J = 2.3, 12.5 Hz, H-6), 3.58 (1H, dd, J = 5.7, 12.5 Hz, H-6), 3.21-3.05 (4H, m, H-3, H-4, H-5, and H-1ax), 2.51 (1H, m, H-1'), 1.85 (1H, m, H-2), 1.65 (1H, m, H-1'); ^{13}C NMR (100 MHz, CD_3OD) δ 137.3, 116.9, 82.6, 77.9, 73.3, 70.8, 43.3, 33.4; HRMS calcd for $C_9H_{16}O_4Na$ ($M+Na^+$) 211.0946, found 211.0944

1, 5-Anhydro-4, 6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-D-glucitol **(25).**



To a solution of **28** (580 mg, 3.09 mmol) in dry CH_3CN (40 ml), benzaldehyde dimethylacetal (0.70 ml, 4.67 mmol) and $TsOH{\cdot}H_2O$ (118 mg, 0.62 mmol) were successively added at room temperature. After 3 hours, triethylamine (2.0 ml) was added to the reaction mixture at 0 °C and the solvent was removed by evaporation. The residue was purified by chromatography (EtOAc/hexane, 1:5) to afford **25** (770 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.38 (5H, m), 5.82 (1H, m, H-2'), 5.52 (1H, s), 5.14-5.00 (2H, m, H-3'), 4.27 (1H, dd, *J*= 5.0, 10.3 Hz, H-6), 3.95 (1H, dd, *J* = 4.6, 11.4 Hz, H-1eq), 3.69 (1H, t, *J* =10.3 Hz, H-6), 3.55 (1H, t, *J* = 9.0, Hz, H-3), 3.46 (1H, t, *J* = 9.0 Hz, H-4), 3.34 (1H, m, H-5), 3.23 (1H, t, *J* = 11.4 Hz, H-1ax), 2.59-2.50 (1H, m, H-1'), 2.00-1.85 (2H, m, H-2 and H-1'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.3, 135.3, 129.2, 128.3, 128. 3, 126.2, 126.2, 117.0, 101.9, 83.3, 73.0, 71.2, 70.5, 68.9, 42.1, 32.3; HRMS calcd for $C_{16}H_{20}O_4Na$ ($M+Na^+$) 299.1259, found 299.1259

1, 5-Anhydro-4, 6-O-benzylidene-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(29).**



To a solution of **25** (760 mg, 2.75 mmol) in pyridine (9.0 ml), myristoyl chloride (1.85 ml, 6.81 mmol) was added at 0 °C, and the mixture was stirred for 3 hours at room temperature. EtOAc (30 ml) was added to the reaction mixture and the solution was washed with diluted HCl (5 ml), sat. $NaHCO_3$ (5 ml), and brine (5 ml), dried over Na_2SO_4

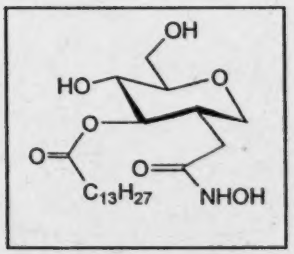
and the residue was purified by chromatography (EtOAc/hexane, 1:7) to give **29** (1.31 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.38 (5H, m), 5.72 (1H, m, H-2'), 5.50 (1H, s), 5.14-5.00 (3H, m, H-3' and H-3), 4.30 (1H, dd, J = 4.9, 10.2 Hz, H-6), 3.99 (1H, dd, J = 4.9, 11.6 Hz, H-1eq), 3.70 (1H, t, J = 10.2 Hz, H-6), 3.56 (1H, t, J = 9.3 Hz, H-4), 3.42 (1H, m, H-5), 3.31 (1H, t, J = 11.6 Hz, H-1ax), 2.32 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.22 (1H, m, H-1'), 2.05 (1H, m, H-2), 1.92 (1H, m, H-1'), 1.60 (2H, m), 1.36-1.12 (20H, m), 0.90 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 137.3, 134.6, 128.8, 128.1, 128.1, 126.0, 117.2, 101.3, 80.8, 73.2, 71.8, 70.6, 68.9, 41.0, 34.4, 32.4, 31.9, 29.6, 29.6, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 25.1, 22.6, 14.1; HRMS calcd for $C_{30}H_{46}O_5Na$ ($M+Na^+$) 509.3243, found 509.3244

1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol **(18).**

To a solution of **29** (200 mg, 0.41 mmol) in $CCl_4/CH_3CN/H_2O$ (2:2:3, 7ml), $NaIO_4$ (396 mg, 1.85 mmol) and $RuCl_3 \cdot H_2O$ (8 mg, 0.04 mmol) were successively added at room temperature. The reaction mixture was stirred vigorously for 1 hour and extracted with CH_2Cl_2 (20 ml x 2). The combined organic layers were dried over Na_2SO_4 and concentrated to afford the acid. This crude product was directly used in the next step. To a solution of the crude acid and $BnONH_2 \cdot HCl$ (56 mg, 0.35 mmol) in CH_2Cl_2 (3 ml) were added successively EDC (87 mg, 0.45 mmol) and triethylamine (63 µl, 0.45 mmol) at room temperature. The reaction mixture was stirred for 2 hours. CH_2Cl_2 (20 ml) was then added and the solution was washed with water (5 ml) and brine (5 ml), then dried over

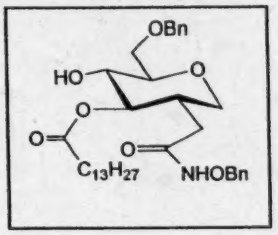
Na_2SO_4. The residue after concentration was purified by chromatography to afford **18** (175 mg, 70%.)

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-myristoyl-D-glucitol **(1).**



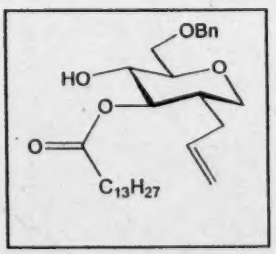
Pd-C (20 mg) was added to a solution of **18** (30 mg, 49 μmol) in AcOH (2 ml). The reaction mixture was stirred for 6 hours under a balloon of H_2. After the filtration through celite and the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **1** (15 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.74 (1H, dd, J = 8.9, 10.0 Hz, H-3), 3.94 (1H, dd, J = 4.7, 11.6 Hz, H-1eq), 3.81 (1H, dd, J = 2.32, 11.8 Hz, H-6), 3.62 (1H, dd, J = 5.7, 11.6 Hz, H-6), 3.38 (1H, t, J = 10.0 Hz, H-4), 3.20 (2H, m, H-5 and H-1ax), 2.38 (1H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.24 (1H, m, H-2), 2.10 (1H, dd, J = 4.9, 14.5 Hz, H-1'), 1.72 (1H, dd, J = 9.1, 14.5 Hz, H-1'), 1.60 (2H, m), 1.30 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 170.3, 82.7, 79.1, 70.7, 70.3, 62.9, 39.8, 35.1, 33.0, 32.7, 30.8, 30.7, 30.4, 30.2, 25.9, 23.7, 14.4; HRMS calcd for $C_{22}H_{41}NO_7Na$ ($M+Na^+$) 454. 2780; found 454.2780

1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol **(32)**



A solution of **18** (50 mg, 82 μmol) and $NaCNBH_3$ (52 mg, 0.82 mmol) in dry THF (2 ml) was added HCl in Et_2O until bubbles ceased. The mixture was diluted with CH_2Cl_2 (20 m l) and water (5 ml), and then poured into sat. $NaHCO_3$ (5 ml). The organic layer was washed with brine (5ml), dried over Na_2SO_4 and then concentrated. The residue was purified by chromatography (EtOAc/hexane, 1:2) to yield **32** (43 mg, 86%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.42-7.25 (10H, m), 4.85 and 4.80 (2H, ABd, J = 11.0 Hz, OCH_2Ph), 4.74 (1H, dd, J = 8.7, 10.9 Hz, H-3), 4.56 (2H, s, OCH_2Ph), 3.86 (1H, dd, J = 4.7, 11.6 Hz, H-1eq), 3.77 (1H, dd, J = 1.9, 10.9 Hz, H-6), 3.64 (1H, dd, J = 5.4, 10.9 Hz, H-6), 3.42 (1H, t, J = 8.7 Hz, H-4), 3.38 (1H, m H-5), 3.20 (1H, t, J = 11.6 Hz, H-1ax), 2.38 (2H, t, J = 7.5 Hz, $OC(O)CH_2$), 2.26 (1H, m, H-2), 2.08 (1H, dd, J = 5.0, 14.7 Hz, H-1′), 1.82 (1H, dd, J = 8.7, 14.7 Hz, H-1′), 1.61 (2, m), 1.40-1.20 (20H, m, $C_{10}H_{20}$), 0.9 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 170.2, 139.6, 136.8, 130.3, 129.7, 129.6, 129.4, 128.9, 128.7, 81.7, 79.0, 78.9, 74.5, 71.0, 70.8, 70.4,39.7, 35.1, 33.0, 32.6, 30.8, 30.6, 30.5, 30.3, 25.9, 23.7, 14.5; HRMS calcd for $C_{36}H_{53}NO_7Na$ ($M+Na^+$) 634.3714, found 634.3712

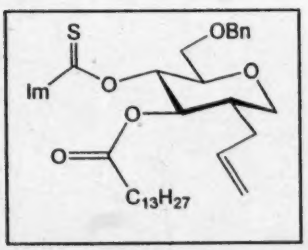
1,5-Anhydro-6-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(40)**



To a solution of **29** (160 mg, 0.33 mmol) and $NaCNBH_3$ (207 mg, 3.29 mmol) in dry THF (6 ml), HCl in Et_2O was added until bubbles ceased. The mixture was diluted with CH_2Cl_2 (20 m l) and water (5 ml), poured into sat. $NaHCO_3$ (5 ml). The organic layer was washed with brine (5ml), dried over Na_2SO_4 and then concentrated. The

residue was purified by chromatography (EtOAc/hexane, 1:5) to yield **40** (131 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.25 (5H, m), 5.79 (1H, m, H-2′), 5.10-4.90 (2H, m, H-3′), 4.72 (1H, dd, J = 9.0, 10.6 Hz. H-3), 4.60 and 4.58 (2H, ABd, J = 12.0 Hz, OCH_2Ph), 3.96 (1H, dd, J = 4.7, 11.9 Hz, H-1eq), 3.71 (2H, m, H-6), 3.55 (1H, m, H-4), 3.39 (1H, m, H-5), 3.15 (1H, t, J = 11.9 Hz, H-1ax), 2.36 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.18 (1H, m, H-1′), 1.95 (1H, m, H-2), 1.86 (1H, m, H-1′), 1.63 (2H, m), 1.36-1.12 (20H, m), 0.90 (3H, t, J = 6.7 Hz) ;^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 137.8, 134.6, 128.4, 127.7, 117.1, 79.5, 78.4, 73.7, 71.7, 70.3, 69.8, 40.1, 34.4, 32.3, 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 24.9, 22.6, 14.1; HRMS calcd for $C_{30}H_{48}O_4Na$ ($M+Na^+$) 511.3399, found 511.3401

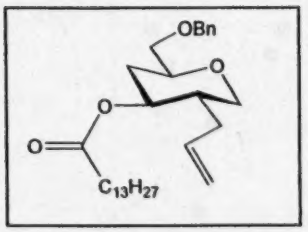
1,5-Anhydro-6-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-4-O-(1-thiocarbonylimidazol)-D-glucitol **(41)**



A solution of **40** (91 mg, 0.19 mmol) and N,N-thiocarbonyldiimidazole (110 mg, 0.62 mmol) in dry toluene (4 ml) was refluxed overnight, After cooling to room temperature, the mixture was evaporated and the residue was purified directly by chromatography (EtOAc/hexane, 1:4) to give **41** (85 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, s), 7.50 (1H, s), 7.24-7.18 (5H, m), 7.00 (1H, s), 5.79 (1H, t, J = 9.5 Hz, H-4), 5.69 (1H, m, H-2′), 5.16 (1H, dd, J = 9.2, 10.7 Hz, H-3), 5.05-4.98 (2H, m, H-3′), 4.46 (2H, s, OCH_2Ph), 4.06 (1H, dd, J = 4.6, 11.5 Hz, H-1eq), 3.70 (1H, m, H-5), 3.58 (1H, dd, J = 3.2, 10.7 Hz, H-6), 3.51 (1H, dd, J = 5.1, 10.7 Hz), 3.38 (1H, t, J = 11.5 Hz, H-1ax), 2.24-2.10 (4H, m, H-2, H-1′, $OC(O)CH_2$), 1.90 (1H, m,

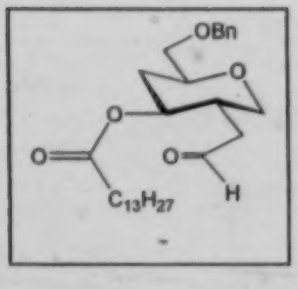
H-1′), 1.40 (2H, m), 1.38-1.00 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.5, 173.2, 134.0, 130.9, 128.2, 127.8, 127.7, 117.5, 79.3, 77.7, 74.4, 73.7, 69.9, 69.2, 40.6, 34.1, 32.1, 31.9, 29.6, 29.5, 29.3, 28.9, 24.7, 22.6, 14.1; HRMS calcd for $C_{34}H_{50}N_2O_5SH$ ($M+H^+$) 599.3519, found 599.3518

1,5-Anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(42)**



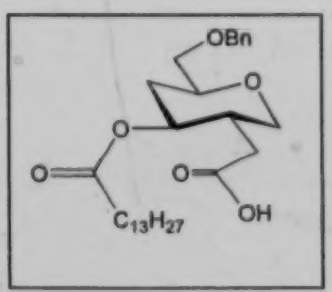
To a solution of **41** (63 mg, 0.11 mmol) and AIBN (5 mg, 0.04 mmol) in dry toluene (3 ml) was added Bu_3SnH (85 ul, 0.33 mmol) under argon. The mixture was refluxed for 3 hours. After cooling to room temperature, the mixture was concentrated. Column chromatography (EtOAc/hexane, 1:6) of the residue gave **42** (44 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.24 (5H, m), 5.70 (1H, m, H-2′), 5.05-4.95 (2H, m, H-3′), 4.70 (1H, m, H-3), 4.56 and 4.54 (2H, ABd, J = 12.3 Hz, OCH_2Ph), 4.04 (1H, dd, J = 4.2, 11.8 Hz, H-1eq), 3.61 (1H, m, H-5), 3.94-3.80 (2H, m, H-6), 3.38 (1H, t, J = 11.8 Hz, H-1ax), 2.28 (2H, t, J = 7.3 Hz, $OC(O)CH_2$), 2.25 (1H, m, H-4), 1.98 (1H, m, H-1′), 1.86-1.78 (2H, m, H-2, H-1′), 1.60 (2H, m), 1.39 (1H, q, J = 11.6 Hz, H-4), 1.34-1.18 (20H, m), 0.90 (3H, t, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 137.9, 135.0, 128.3, 127.6, 116.7, 75.5, 73.5,73.4, 72.8, 70.3, 40.7, 34.6, 34.1, 32.7, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 25.1, 22.8, 14.2; HRMS calcd for $C_{30}H_{48}O_4Na$ ($M+Na^+$) 495.3450, found 495.3456

2-C-(Aldehydomethyl)-1,5-anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-D-glucitol **(43)**



Ozone was passed through a solution of **42** (48 mg, 0.10 mmol) in CH_2Cl_2 (10 ml) at –78 °C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 minutes, followed by addition of Ph_3P (53 mg, 0.20 mmol). The mixture was allowed to warm to room temperature for 2 hours, and then concentrated. The residue was purified by chromatography (EtOAc/hexane, 1:3) to afford **43** (42 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (1H, s), 7.38-7.20 (5H, m), 4.67 (1H, m, H-3), 4.56 and 4.52 (2H, ABd, J = 12.3 Hz, OCH_2Ph), 4.04 (1H, dd, J = 4.5, 11.8 Hz, H-1eq), 3.63 (1H, m, H-5), 3.50-3.40 (2H, m, 2H-6), 3.19 (1H, t, J = 11.8 Hz, H-1ax), 2.45-2.32 (2H, m, H-1′, H-2), 2.23 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.14 (1H, m, H-4), 2.02 (1H, m, H-1′), 1.60 (2H, m), 1.44 (1H, q, J = 11.7 Hz, H-4), 1.34-1.18 (20H, m), 0.86 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.9, 173.1, 137.8, 128.3, 127.6, 75.5, 73.5, 73.4,72.6, 70.0, 42.9, 36.4, 34.4, 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 24.9, 22.8, 14.2

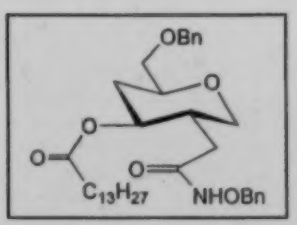
2-C-(Carboxymethyl)-1,5-anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-D-glucitol **(44)**



To a solution of **43** (38 mg, 0.08 mmol) in *t*-BuOH (2 ml) and 2-methyl-2-butene (0.6 ml) was added a solution of $NaClO_2$ (91 mg, 0.80 mmol) and NaH_2PO_4 (138 mg, 0.80 mmol) in water (1.5 ml) over 5 minutes. The mixture was stirred for 1 hour, then diluted with ice water, and extracted with EtOAc. The combined organic extracts were washed with brine (5 ml), dried over

Na_2SO_4, evaporated and further dried under oil vacuum overnight to afford **44**. The acid was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.20 (5H, m), 4.67 (1H, m, H-3), 4.56 and 4.52 (2H, ABd, J = 12.3 Hz, OCH_2Ph), 4.04 (1H, dd, J = 4.5, 11.8 Hz, H-1eq), 3.63 (1H, m, H-5), 3.50-3.40 (2H, m, 2H-6), 3.19 (1H, t, J = 11.8 Hz, H-1ax), 2.45-2.32 (1H, m, H-1′), 2.23 (3H, m, $OC(O)CH_2$,H-2), 2.10-2.00 (2H, m, H-4, H-1′), 1.60 (2H, m), 1.44 (1H, q, J = 11.7 Hz, H-4), 1.34-1.18 (20H, m), 0.86 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 173.4, 137.8, 129.7, 128.4, 127.7, 127.6, 75.5, 73.4, 73.0, 72.5, 69.9, 38.0, 34.3, 33.8, 32.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 24.9, 22.8, 14.2

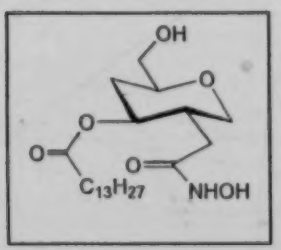
1, 5-Anhydro-6-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,4-dideoxy-3-O-myristoyl-D-glucitol **(45)**



To a solution of the acid **44** (40 mg, 0.08 mmol) and $BnONH_2$·HCl (13 mg, 0.08 mmol) in CH_2Cl_2 (0.5 ml) were added successively EDC (19 mg, 0.10 mmol) and triethylamine (13 µl, 0.10 mmol) at room temperature. The reaction mixture was stirred for 2 hours. CH_2Cl_2 (10 ml) was then added and the solution was washed with water (5 ml) and brine (5 ml), then dried over Na_2SO_4. The residue after concentration was purified by chromatography (toluene/EtOAc, 4:1) to afford **45** (34 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.20 (10H, m), 4.84 and 4.80 (2H, ABd, J = 11.2 Hz, OCH_2Ph), 4.70 (1H, m, H-3), 4.53 (2H, s, OCH_2Ph), 3.93 (1H, dd, J = 4.3, 11.5 Hz, H-1eq), 3.62 (1H, m H-5), 3.52-3.42 (2H, m, H-6), 3.18 (1H, t, J = 11.3 Hz, H-1ax), 2.30 (2H, m, $OC(O)CH_2$), 2.20-2.10 (2H, m, H-2, H-1′), 2.02 (1H, m, H-4), 1.80

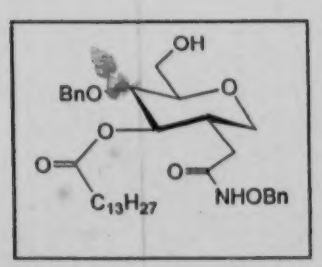
(1H, dd, J =7.2, 13.8 Hz, H-1′), 1.58 (2H, m), 1.35 (1H, q, J = 11.5 Hz, H-4), 1.33-1.20 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 170.7, 139.5, 136.9, 130.3, 129.7, 129.5, 129.4, 128.9, 128.7, 79.0, 76.7, 74.8, 74.4, 73.9, 70.8, 39.7, 35.2, 33.1, 32.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.9, 23.8, 14.5; HRMS calcd. for $C_{36}H_{53}NO_6Na$ ($M+Na^+$) 618.3765, found 618.3762

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,4-dideoxy-3-O-myristoyl-D-glucitol **(2)**



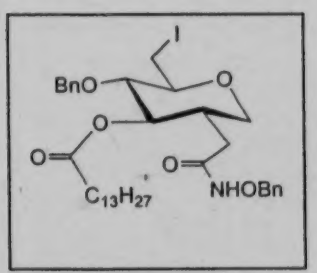
Pd/C (8 mg) was added to a solution of **45** (12 mg, 20 μmol) in AcOH (2 ml). The reaction mixture was stirred for 4 hours under a balloon of H_2. After filtration through celite, the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **2** (5.9 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.71 (1H, m, H-3), 4.00 (1H, dd, J = 4.3, 11.5 Hz, H-1eq), 3.52-3.44 (3H, m, 2H-6, H-5), 3.22 (1H, t, J = 11.5 Hz, H-1ax), 2.33 (2H, $OC(O)CH_2$), 2.22-2.15 (2H, H-2, H-1′), 2.02 (1H, m, H-4), 1.83 (1H, dd, J = 9.7, 15.7 Hz, H-1′), 1.61 (2H, m), 1.38-1.26 (21H, m, H-4, $C_{10}H_{20}$), 0.90 (3H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 170.7, 78.3, 75.1, 70.9, 65.9, 39.8, 35.1, 34.8, 33.1, 32.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.9, 23.7, 14.4; HRMS calcd for $C_{22}H_{41}NO_6Na$ ($M+Na^+$) 438.2832, found 438.2829

1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol **(46)**



A solution of 1M BH_3 in THF (0.2 ml) was added to **18** (12 mg, 20 μmol) at 0 °C, and then a solution of 1M Bu_2BOTf in CH_2Cl_2 (0.02 ml, 20 μmol) was added to the reaction slowly. After 1 hour at 0 °C, triethylamine (0.1 ml) was then added followed by careful addition of methanol until the evolution of H_2 ceased. The reaction mixture was then concentrated and purified by chromatography (toluene/EtOAc, 2:1) to give **46** (8.5 mg, 71%). ^{1}H NMR (500 MHz, CD_3OD: $CDCl_3$ = 4:1) δ 7.40-7.20 (10H, m), 4.92 (1H, dd, J = 9.1, 10.9 Hz, H-3), 4.80 (2H, overlapped with H_2O, OCH_2Ph), 4.64 and 4.58 (2H, 2d, J = 11.3 Hz, OCH_2Ph), 3.89 (1H, dd, J = 4.8, 11.6 Hz, H-1 eq), 3.80 (1H, dd, J = 2.0, 12.0 Hz, H-6), 3.67 (1H, dd, J = 4.6, 12.0 Hz, H-6), 3.54 (1H, t, J = 9.1 Hz, H-4), 3.36 (1H, m, H-5), 3.19 (1H, t, J = 11.6 Hz, H-1ax), 2.28 (2H, m, $OC(O)CH_2$), 2.16 (1H, m, H-2), 2.06 (1H, dd, J = 5.0, 14.8 Hz, H-1'), 1.81 (1H, dd, J = 8.7, 14.8 Hz, H-1'), 1.52 (2H, m), 1.34-1.18 (20H, m), 0.88 (1H, t, J = 6.8 Hz); ^{13}C NMR (125 MHz, CD_3OD : $CDCl_3$ = 4:1) δ 175.4, 170.2, 139.5, 136.8, 130.3, 129.7, 129.5, 129.4, 128.8, 128.7, 81.9, 79.4, 79.1, 78.5, 78.4, 75.7, 70.4, 62.4, 40.0, 35.2, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 25.8, 23.8, 14.6; HRMS calcd for $C_{36}H_{53}NO_7Na$ ($M+Na^+$) 634.3720, found 634.3721

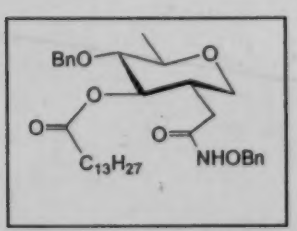
1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,6-dideoxy-6-iodine-3-O-myristoyl-D-glucitol **(47)**



To a solution of **46** (12 mg, 20 μmol) in toluene (1 ml) were added Ph_3P (10.3 mg, 40 μmol), I_2 (10 mg, 0.040 mmol) and imidazole (5.3 mg, 78 μmol) successively. The mixture was

stirred at room temperature for 4 hours. It was then directly purified by chromatography (EtOAc/toluene, 1:2) to give **47** (11 mg, 80%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.20 (10H, m), 4.99 (1H, dd, J = 8.9, 10.9 Hz, H-3), 4.83 (2H, overlapped with H_2O, OCH_2Ph), 4.72 and 4.65 (2H, ABd, J = 11.3 Hz, OCH_2Ph), 3.86 (1H, dd, J = 4.8, 11.7 Hz, H-1 eq), 3.50 (1H, dd, J = 2.8, 10.9 Hz, H-6), 3.42 (1H, t, J = 8.9 Hz, H-4), 3.40 (1H, dd, J = 10.9, 5.2 Hz, H-6), 3.27 (1H, t, J = 11.7 Hz, H-1ax), 3.01 (1H, m, H-5), 2.28 (2H, m, $OC(O)CH_2$), 2.19 (1H, m, H-2), 2.06 (1H, dd, J = 5.1, 14.8 Hz, H-1′), 1.81 (1H, dd, J = 8.5, 14.8 Hz, H-1′), 1.52 (2H, m), 1.35-1.18 (20H, m), 0.90 (1H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 170.1, 139.5, 130.3, 129.7, 129.5, 129.4, 128.9, 128.7, 82.5, 79.5, 78.9, 77.9, 76.1, 70.1, 40.1, 35.1, 33.1, 32.3, 30.7, 30.5, 30.4, 30.2, 25.7, 23.7, 7.6; HRMS calcd for $C_{36}H_{52}NO_6INa$ ($M+Na^+$) 744.2737, found 744.2730

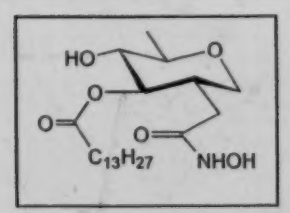
1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,6-dideoxy-3-O-myristoyl-D-glucitol **(48)**



A solution of **47** (17 mg, 24 μmol) and AIBN (1.5 mg, 9.0 μmol) in dry toluene (0.5 ml) was degassed with Argon for 10 minutes. Bu_3SnH (25 μl, 96 μmol) was then added under Argon. The mixture was heated up to 90 °C for 30 minutes. After cooling to room temperature, it was directly purified by chromatography (EtOAc/toluene, 1:2) to give 48 (11.5 mg, 82%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.20 (10H, m), 4.89 (1H, dd, J = 9.0, 10.9 Hz, H-3), 4.82 (2H, overlapped with H_2O, OCH_2Ph), 4.62 and 4.60 (2H, ABd, J = 11.4 Hz, OCH_2Ph), 3.79 (1H, dd, J = 4.8, 11.7 Hz, H-1 eq), 3.36-3.30 (2H, m, H-4, H-5), 3.17 (1H, t, J = 11.7 Hz, H-1ax), 2.28 (2H, m,

$OC(O)CH_2$), 2.18 (1H, m, H-2), 2.06 (1H, dd, J = 5.1, 14.8 Hz, H-1'), 1.81 (1H, dd, J = 8.7, 14.8 Hz, H-1'), 1.52 (2H, m), 1.35-1.18 (22H, m, H-6 and $C_{10}H_{20}$), 0.89 (1H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 170.2, 139.6, 136.9, 130.8, 130.2, 129.6, 129.4, 129.3, 128.7, 128.6, 84.5, 79.0, 78.2, 77.6, 75.9, 70.3, 40.4, 35.2, 33.2, 32.6, 30.9, 30.8, 30.6, 30.5, 30.3, 25.9, 23.9, 18.9, 14.6; HRMS calcd for $C_{36}H_{53}NO_6Na$ ($M+Na^+$) 618.3765, found 618.3761

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,6-dideoxy-3-O-myristoyl-D-glucitol **(3)**

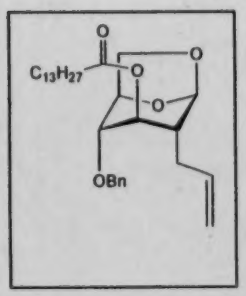


Pd/C (7 mg) was added to a solution of **48** (12 mg, 20 μmol) in AcOH (2 ml). The reaction mixture was stirred for 4 hours under a balloon of H_2. After filtration through celite, the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **3** (5.9 mg, 70%). ^1H NMR (400 MHz, CD_3OD) δ 4.70 (1H, dd, J = 9.0, 10.9 Hz, H-3), 4.00 (1H, dd, J = 4.7, 11.6 Hz, H-1eq), 3.25 (1H, m, H-5), 3.20 (1H, t, J = 11.6 Hz, H-1ax), 3.09 (1H, t, J = 9.0 Hz, H-4), 2.39 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.25 (H, m, H-2), 2.11 (1H, dd, J = 4.9, 10.0 Hz H-1'), 1.82 (1H, dd, J = 10.0, 15.7 Hz, H-1') 1.65 (2H, m), 1.40-1.25 (20H, m, $C_{10}H_{20}$), 1.24 (3H, d, J = 6.1 Hz, 2H-6), 0.90 (3H, t, J = 6.8 Hz); **(No ^{13}C-data)**

After sitting overnight in the NMR machine, ^1H NMR (400 MHz, CD_3OD) δ 4.70 (1H, dd, J = 9.0, 10.9 Hz, H-3), 4.54 (0.4 H, t, J = 9.0 Hz, H-4), 4.00 (1H, dd, J = 4.7, 11.6 Hz, H-1eq), 3.25 (1H, m, H-5), 3.20 (1H, t, J = 11.6 Hz, H-1ax), 3.09 (1H, t, J = 9.0 Hz, H-4),

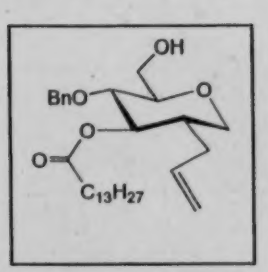
2.39 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.25 (H, m, H-2), 2.11 (1H, dd, J = 4.9, 10.0 Hz H-1'), 1.82 (1H, dd, J = 10.0, 15.7 Hz, H-1') 1.65 (2H, m), 1.40-1.25 (20H, m, $C_{10}H_{20}$), 1.24 (3H, d, J = 6.1 Hz, 2H-6), 1.10 (d, J = 6.1 Hz, 2H-6), 0.90 (3H, t, J = 6.8 Hz); HRMS calcd for $C_{22}H_{41}NO_6Na$ ($M+Na^+$) 438.2832, found 438.2829

1,6-Anhydro-4-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-β-D-glucopyranose **(49)**



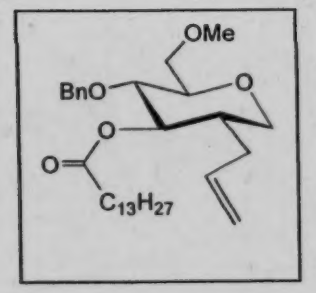
To a solution of **14** (340 mg, 1.23 mmol) in pyridine (8 ml), DMAP (70 mg, 0.57 mmol) and myristoyl chloride (0.86 ml, 3.16 mmol) were successively added at 0 °C and then stirred at room temperature for 20 hours. EtOAc (20 ml) was added to the reaction mixture and the organic layer was washed with 10% citric acid (5 ml), H_2O (5ml x 2). sat. $NaHCO_3$ (5 ml), and brine (5 ml). The organic layer was dried over Na_2SO_4, and concentrated. The residue was purified by chromatography (toluene/EtOAc, 9:1) to afford **49** (503 mg, 84%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.22 (5H, m), 5.80 (1H, m, H-2'), 5.49 (1H, s, H-1), 5.14-5.08 (2H, m, H-3'), 4.90 (1H, s, H-3), 4.80 (1H, d, J = 12.3 Hz, OCH_2Ph), 4.65 (1H, d, J = 12.3 Hz, OCH_2Ph), 4.54 (1H, br d, J = 5.6 Hz, H-5), 3.96 (1H, d, J = 7.0 Hz, H-6), 3.70 (1H, dd, J = 5.6, 7.0 H, H-6), 3.22 (1H, s, H-4), 2.40 –2.26 (4H, m, 2H-1' and $OC(O)CH_2$), 1.79 (1H, br t, J = 8.0 Hz, H-2), 1.38-1.20 (20H, m), 0.82 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 137.7, 135.6, 128.4, 127.8, 127.7, 117.4, 102.4, 75.5, 74.7, 71.1, 69.1, 64.8, 42.6, 34.5, 33.9, 31.9, 29.6, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1; HRMS calcd for $C_{30}H_{46}O_5Na$ ($M+Na^+$) 509.3243, found 509.3243

1,5-Anhydro-4-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(50)**



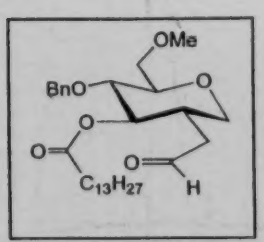
To a solution of **49** (100 mg, 0.21 mmol) in CH_2Cl_2 (20 ml), Et_3SiH (0.16 ml, 1.02 mmol) and $SnCl_4$ (36 µl, 0.31 mmol) were successively added at 0 °C. After stirring at room temperature for 1 hour, the reaction mixture was poured into cold sat. $NaHCO_3$ (5ml) and the aqueous layer was extracted with CH_2Cl_2 (10 ml x 2) at once. The combined organic layers were washed with brine (5ml) and dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 6:1) to give **50** (46 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.20 (5H, m), 5.70 (1H, m, H-2′), 5.06-4.95 (3H, m, H-3, H-3′), 4.61 and 4.59 (2H, ABd, J = 11.3 Hz, OCH_2Ph), 3.94 (1H, dd, J =4.3, 11.6 Hz, H-1 eq), 3.85 (1H, dd, J = 2.6, 11.9 Hz, H-6), 3.70 (1H, dd, J = 4.0, 11.9 Hz, H-6), 3.52 (1H, t, J = 9.4 Hz, H-4), 3.30 (1H, m, H-5), 3.18 (1H, t, J = 11.6 Hz, H-1ax), 2.31 –2.10 (3H, m, H-1′, $OC(O)CH_2$), 1.95-1.80 (2H, m, H-2, H-1′), 2.50 (2H, m), 1.38-1.20 (20H, m), 0.84 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 137.9, 134.8, 128.4, 127.7, 127.6, 116.9, 79.8, 77.1, 74.5, 69.7, 62.1, 41.2, 34.4, 32.2, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1; HRMS calcd for $C_{30}H_{48}O_5Na$ ($M+Na^+$) 511.3399, found 511.3399

1,5-Anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(51)**



To a solution of **50** (70 mg, 0.14 mmol) and 2,6-di (*tert*-butyl-4-methyl) pyridine (882 mg, 4.30 mmol) was added methyl trifluoromethanesulfonate (0.32 ml, 2.84 mmol). The mixture was stirred overnight and quenched by an aqueous ammonia solution (5 ml), and extracted with CH_2Cl_2 (10 ml x 3). The extracts were washed with 10% HCl (5 ml), brine (5 ml) and dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 8:1) to afford **51** (71 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.20 (5H, m), 5.70 (1H, m, H-2′), 5.06-4.95 (3H, m, H-3, H-3′), 4.61 and 4.59 (2H, ABd, J = 11.3 Hz, OCH_2Ph), 3.94 (1H, dd, J = 4.7, 11.7 Hz, H-1 eq), 3.58-3.53 (3H, m, 2H-6, H-4), 3.40-3.35 (4H, m, H-5, OMe), 3.25 (1H, t, J = 11.7 Hz, H-1ax), 2.31 –2.10 (3H, m, H-1′, $OC(O)CH_2$), 2.00-1.80 (2H, m, H-2, H-1′), 2.50 (2H, m), 1.38-1.20 (20H, m), 0.84 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 138.1, 134.9, 128.3, 127.6, 127.5, 116.8, 79.2, 77.2, 74.5, 71.4, 69.9. 59.2, 41.1, 34.4, 32.2, 31.8, 29.6, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1; HRMS calcd for $C_{31}H_{50}O_5Na$ ($M+Na^+$) 525.3556, found 525.3554

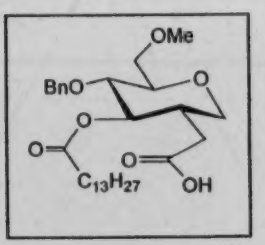
2-C-(Aldehydomethyl)-1,5-anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol **(52)**



Ozone was passed through a solution of **51** (30 mg, 60 μmol) in CH_2Cl_2 at –78 °C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 minutes, followed by addition of Ph_3P (23 mg, 88 μmol). The mixture was allowed to warm to room temperature for 2 hours, concentrated and purified by chromatography

(EtOAc/hexane, 1:3) to afford **52** (24 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (1H, s), 7.38-7.20 (5H, m), 5.00 (1H, dd, J = 9.1, 10.7 Hz, H-3), 4.61 and 4.59 (2H, ABd, J = 14.4 Hz, OCH_2Ph), 3.98 (1H, dd, J = 4.5, 11.4 Hz, H-1eq), 3.64-3.56 (3H, m, 2H-6, H-4), 3.44-3.34 (4H, m, H-5, OMe), 3.21 (1H, t, J = 11.4 Hz, H-1ax), 2.56-2.40 (2H, m, H-2, H-1′), 2.31 –2.10 (3H, m, H-1′, $OC(O)CH_2$), 2.00-1.80 (2H, m, H-2,), 2.50 (2H, m), 1.38-1.20 (20H, m), 0.84 (3H, t, J =6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.4, 173.4, 137.9, 128.4, 127.7, 127.5, 79.4, 76.9, 74.6, 71.3, 69.6, 59.2, 42.2, 36.4, 34.2, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.7, 22.6, 14.1; HRMS calcd for $C_{30}H_{48}O_6Na$ ($M+Na^+$) 527.3349, found 527.3349

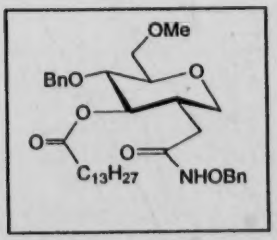
2-C-(Carboxymethyl)-1,5-anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol **(53)**



To a solution of **52** (24 mg, 48 μmol) in *t*-BuOH (1.5 ml) and 2-methyl-2-butene (0.5 ml) was added a solution of $NaClO_2$ (43 mg, 0.48 mmol) and NaH_2PO_4 (66 mg, 0.48 mmol) in water (1 ml). The mixture was stirred for 1 hour, then diluted with ice-water (5 ml), and extracted with EtOAc (10 ml x 3). The combined organic extracts were washed with brine (5 ml), dried over Na_2SO_4, concentrated and dried under high vacuum overnight to give **53**. The acid was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.20 (5H, m), 4.94 (1H, dd, J = 9.0, 10.6 Hz, H-3), 4.61 and 4.59 (2H, ABd, J = 14.4 Hz, OCH_2Ph), 3.98 (1H, dd, J = 4.7, 11.5 Hz, H-1 eq), 3.64-3.56 (3H, m, 2H-6, H-4), 3.40-3.32 (4H, m, H-5, OMe), 3.21 (1H, t, J = 11.5 Hz), 2.56-2.40 (2H, m,

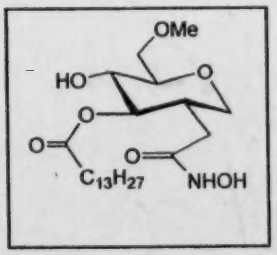
H-2, H-1'), 2.31 –2.10 (3H, m, H-1', $OC(O)CH_2$), 2.50 (2H, m), 1.38-1.20 (20H, m), 0.84 (3H, t, J =6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 173.5, 137.9, 128.5, 128.4, 127.7, 127.6, 79.3, 76.9, 76.5, 74.6, 71.3, 69.6, 59.2, 38.4, 34.3, 32.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 291, 24.7, 22.6, 14.1

1, 5-Anhydro-4-O-benzyl-2-deoxy-2-C-(carboxymethyl N-hydroxyamide)-6-O-methyl-3-O-myristoyl-D-glucitol **(54)**



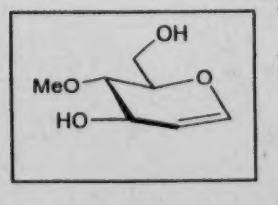
To a solution of the acid **53** (24 mg, 50 μmol) and $BnONH_2 \cdot HCl$ (7.6 mg, 50 μmol) in CH_2Cl_2 (0.3 ml) were added successively EDC (12.8 mg, 70 μmol) and triethylamine (9 μl, 70 μmol) at room temperature. The reaction mixture was stirred for 2 hours. CH_2Cl_2 (10 ml) was then added and the solution was washed with water (5 ml) and brine (5 ml), then dried over Na_2SO_4. After concentration, the residue was purified by chromatography (toluene/EtOAc, 4:1) to afford **54** (21 mg, 71%). ^1H NMR (400 MHz, CD_3OD) δ 7.40-7.20 (10H, m), 4.92 (1H, dd, J = 9.0, 10.9 Hz, H-3), 4.93 and 4.80 (2H, ABd, J = 11.3 Hz, OCH_2Ph), 4.60 and 4.58 (1H, dd, J = 4.8, 11.5 Hz, H-1eq), 3.62-3.54 (2H, m, 2H-6), 3.53 (1H, t, J = 9.0 Hz, H-4), 3.36 (1H, m, H-5), 3.34 (3H, s, OMe), 3.29 (1H, t, J = 11.5 Hz, H-1 ax), 2.28 (2H, m, $OC(O)CH_2$), 2.17 (1H, m, H-2), 2.06 (1H, dd, J = 5.1, 14.8 Hz, H-1'), 1.82 (1H, dd, J = 8.6, 14.8 Hz, H-1'), 1.51 (2H, m), 1.34-1.18 (20H, m), 0.89 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 170.2, 139.6, 136.9, 130.3, 129.7, 129.5, 129.4, 126.7, 80.8, 79.0, 78.6, 78.3, 75.7, 72.7, 70.4, 59.5, 40.0, 35.1, 33.1, 32.4, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.7, 23.7, 14.1; HRMS calcd for $C_{37}H_{55}NO_7Na$ ($M+Na^+$) 648.3876, found 648.3874

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol **(4)**



Pd/C (8 mg) was added to a solution of **54** (12 mg, 19 µmol) in AcOH (2 ml), The reaction mixture was stirred for 3 hours under a balloon of H_2. After filtration through celite, the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/ MeOH, 15:1) to give **4** (5.9 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.73 (1H, dd, J = 8.7, 10.9 Hz, H-3), 3.91 (1H, dd, J = 4.8, 11.7 Hz, H-1eq), 3.64 (1H, dd, J = 2.0, 10.8 Hz, H-6), 3.53 (1H, dd, J = 5.4, 10.8 Hz, H-6), 3.40-3.30 (2H, m, H-4, H-5), 3.20 (1H, t, J = 11.7 Hz, H-1ax), 2.38 (2H, t, J = 7.3, $OC(O)CH_2$), 2.24 (1H, m, H-2), 2.09 (1H, dd, J= 4.9, 14.5 Hz, H-1′), 1.81 (1H, dd, J= 9.0, 14.5 Hz H-1′), 1.51 (2H, m), 1.40-1.20(20H, m), 0.9 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 170.3, 81.4, 78.9, 73.3, 70.7, 70.4, 59.5, 39.8, 35.1, 33.1, 32.6, 30.8, 30.4, 30.6, 30.4, 30.2, 25.9, 23.7, 14.4; HRMS calcd for $C_{23}H_{43}NO_7$ ($M+Na^+$) 468.2937, found 468.2936

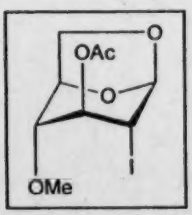
4-O-Methyl-D-glucal **(57)**



A solution of sodium metal in 500 ml ammonia was stirred for 1 hour at –78 °C and then distilled to another 3-neck round bottom flask. Small pieces of sodium metal were added until a deep blue color persisted at –78 °C. A solution of **56** (1.16 g, 3.40 mmol) and MeOH (0.276 ml, 6.80 mmol) in THF (50 ml) was added dropwise to the above

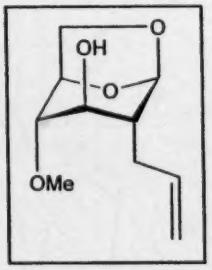
solution. The reaction mixture was stirred for 2 hours, and then quenched with MeOH until the solution turned clear. Ammonia was then evaporated and coevaporated with MeOH and AcOH. The residue was purified by chromatography (EtOAc/acetone, 1:1) to give **57** (464 mg, 85%). The NMR data was the same as that described in the literature [51].

3-O-Acetyl-1, 6-anhydro-2-deoxy-2-iodo-4-O-methy-β-D-glucopyranose **(58)**



The glucal **57** (60 mg, 0.38 mmol) was treated with *bis*-(tributyltin)oxide (0.15 ml, 0.29 mmol) and actived powdered 3Å molecular sieves (200 mg) in refluxing dry acetonitrile (5 ml) for 3 hours. The mixture was cooled to 0 °C under argon, and iodine (143 mg, 0.563 mmol) was added in one portion. The dark-brown mixture was stirred at 0 °C for 15 minutes, then 3 hours at room temperature. The mixture was filtered through celite and concentrated. To the residue was added aq. sodium thiosulfate (10 ml) and it was extracted with EtOAc (20 ml x 3). The combined organic layers were dried over Na_2SO_4 and concentrated, further dried under oil vacuum overnight. The residue was then treated with Ac_2O (3 ml) and pyridine (3 ml) overnight. After removing the solvent, the residue was purified by chromatography (EtOAc/hexane, 1:3) to afford **58** (110 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (1H, s, H-1), 5.39 (1H, s, H-3), 4.54 (1H, d, J = 5.8 Hz, H-5), 4.08 (1H, d, J = 7.6 Hz, H-6), 3.90 (1H, s, H-2), 3.76 (1H, dd, J = 5.8, 7.6 Hz, H-6), 3.50 (3H, s, OMe), 3.16 (1H, s, H-4), 2.08 (3H, s); ^{13}C NMR (125MHz, $CDCl_3$) δ 169.2, 102.5, 78.2, 74.1, 71.3, 65.5, 57.4, 21.5, 21.0; HRMS calcd for $C_9H_{13}O_5INa$ ($M+Na^+$) 350.9700, found 350.9697

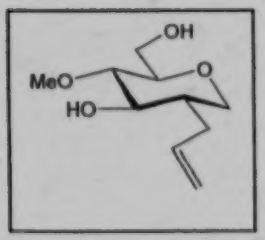
1, 6-Anhydro-2-deoxy-4-O-methyl -2-C- (2-propenyl)-β-D-glucopyranose **(59)**



A solution of **58** (110 mg, 0.34 mmol) and AIBN (20 mg, 0.12 mmol) in dry toluene (6 ml) was degassed with argon for 15 minutes. Allyltributylstannane (0.21 ml, 0.68 mmol) was added under argon, and the mixture was heated to 80 °C for 3 hours. TLC (EtOAc/hexane, 1:1) showed complete conversion. The solvent was evaporated and a solution of the residue in acetonitrile (10 ml) was washed with hexane (10 ml), and then concentrated. Chromatography of the residue gave 56 mg (69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (1H, m, H-2′), 5.36 (1H, s, H-1), 5.18-5.04 (2H, m, H-3′), 4.80 (1H, s, H-3), 4.55 (1H, d, J = 5.8 Hz, H-5), 4.00 (1H, d, J = 8.2 Hz, H-6), 3.75 (1H, dd, J = 5.9, 7.5 Hz, H-6), 3.46 (3H, s, OMe), 3.08 (1H, br s, H-4), 2.28 (2H, t, J = 7.8 Hz, H-1′), 2.05 (3H, s, OAc), 1.76 (1H, br t, J= 8.0 Hz, H-2)

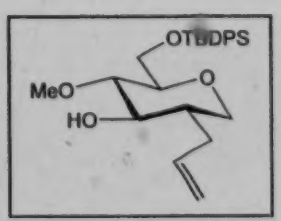
A solution of above compound in 10:10:1 MeOH-H_2O-Et_3N (5 ml) was left overnight at room temperature and then concentrated, dried under oil vacuum overnight to give **59**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (1H, m, H-2′), 5.42 (1H, s, H-1), 5.10-5.06 (2H, m, H-3′), 4.60 (1H, d, J = 5.3 Hz, H-5), 4.10 (1H, d, J = 7.5 Hz, H-6), 3.72 (1H, dd, J = 5.3, 7.5 Hz, H-6), 3.66 (1H, br s, H-3), 3.44 (3H, s, OMe), 3.20 (1H, br s, H-4), 2.28-2.20 (2H, m, H-1′), 1.78 (1H, br t, J= 8.4, H-2); ^{13}C NMR (100MHz, $CDCl_3$) δ 135.8, 117.2, 103.4, 81.5, 74.4, 68.5, 65.4, 57.4, 45.7, 33.5; HRMS calcd for $C_{10}H_{16}O_4Na$ (M+Na+) 223.0946, found 223.0947

1, 5-Anhydro-2-deoxy-4-O-methyl-2-C-(2-propenyl)-D-glucitol **(60)**



To a solution of **59** (196 mg, 0.98 mmol) and Et_3SiH (0.82 ml, 5.15 mmol) in CH_2Cl_2 (50ml), $SnCl_4$ (1.55 ml, 1M in CH_2Cl_2, 1.55 mmol) was dropwise added at 0 ° C. The reaction mixture was stirred at room temperature for 40 minutes. The reaction was then poured into cold sat. $NaHCO_3$ (5 ml) and extracted with CH_2Cl_2 (20 ml x 2). The combined organic layers were washed with brine (5 ml), and dried with Na_2SO_4. The residue was purified by chromatography (acetone/toluene, 1: 6) to give **60** (130 mg, 66%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.81-5.68 (1H, m, H-2′), 5.05-4.98 (2H, m, H-3′), 3.90-3.80 (2H, m, H-6, H-1eq), 3.68 (1H, dd, J = 4.4, 11.8 Hz, H-6), 3.54 (2H, s OMe), 3.34 (1H, dd, J = 8.7, 9.9 Hz, H-3), 3.15 (1H, m, H-5), 3.10-3.02 (2H, m, H-1ax, H-4), 2.48 (1H, m, H-1′), 1.89 (1H, m, H-2), 1.78 (1H, m, H-1′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 116.9, 81.6, 79.7, 76.5, 69.7, 62.3, 60.8, 42.1, 32.4; HRMS calcd for $C_{10}H_{18}O_4Na$ ($M+Na^+$) 225.1103, found 225.1103

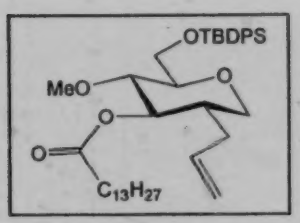
1, 5-Anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-2-C-(2-propenyl)-D-glucitol **(61)**



To a solution of **60** (72 mg, 0.36 mmol) and imidazole (48 mg, 0.71 mmol) in DMF (2 ml) was added *tert*-butyldiphenylsilyl chloride (0.14 ml, 0.54 mmol). The mixture was stirred at room temperature for 3 hours, and then diluted with Et_2O (30 ml) and washed with H_2O (5 ml x 2) and brine (5 ml), dried over Na_2SO_4, concentrated and the residue was purified by chromatography (EtOAc/hexane, 1:5) to afford **61** (152 mg,

97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.34 (10H, m), 5.80 (1H, m, H-2′), 5.10-5.00 (2H, m, H-3′), 3.90 (1H, dd, J = 4.7, 11.7 Hz, H-1 eq), 3.88 –3.82 (2H, m, H-6), 3.54 (3H, s, OMe), 3.38-3.26 (2H, m, H-3, H-4), 3.09 (1H, m, H-5), 3.04 (1H, t, J = 11.7 Hz, H-1 ax), 2.48 (1H, m, H-1′), 1.89 (1H, m, H-2), 1.78 (1H, m, H-1′), 1.04 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8, 135.5, 133.8, 133.3, 129.5, 129.4, 127.5, 127.4, 116.6, 81.2, 80.4, 77.3, 69.8, 63.5, 60.7, 42.2, 32.7, 26.9, 19.5; HRMS calcd for $C_{26}H_{36}O_4SiNa$ ($M+Na^+$) 463.2281, found 463.2281

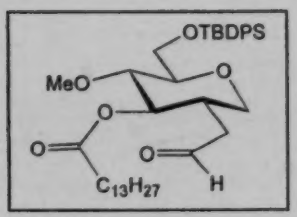
1, 5-Anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol **(62)**



To a solution of **61** (147 mg, 0.33 mmol) in pyridine (5 ml), myristoyl chloride (0.27 ml, 0.99 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 3 hours. EtOAc (20 ml) was added to the reaction mixture. The mixture was washed with H_2O (5 ml x 2), brine (5 ml), dried over Na_2SO_4. The residue was purified by chromatography to give **62** (206 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.34 (10H, m), 5.74 (1H, m, H-2′), 5.02-4.98 (2H, m, H-3′), 4.80 (1H, dd, J = 9.2, 10.4 Hz, H-3), 3.93 (1H, dd, J = 4.3, 11.6 Hz, H-1 eq), 3.88 –3.84 (2H, m, H-6), 3.41 (3H, s, OMe), 3.09 (1H, t, J = 9.2 Hz, H-4), 3.20 (1H, m, H-5), 3.10 (1H, t, J = 11.6 Hz, H-1 ax), 2.38 (2H, t, J = 7.9 Hz, $OC(O)CH_2$), 2.35 (1H, m, H-2), 1.88 (2H, m, H-1′), 1.66 (2H, m), 1.40-1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 135.8, 135.6, 135.2, 133.3, 129.5, 127.6, 127.4, 80.6, 78.6, 77.3, 69.6,

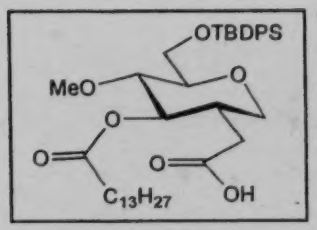
63.3, 60.1, 41.2, 34.6, 32.4, 31.9, 29.6, 29.5, 29.3, 29.2, 26.8, 24.9, 22.4, 19.4, 14.1; HRMS calcd for $C_{40}H_{62}O_5Si$ ($M+Na^+$) 673.4262, found 673.4262

2-C-(Aldehydomethyl)-1, 5-anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl- 2-C-(2-propenyl)-D-glucitol **(63)**



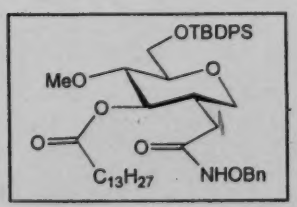
Ozone was passed through a solution of **62** (150 mg, 0.23 mmol) in CH_2Cl_2 (20 ml) at –78 °C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 minutes, followed by addition of Ph_3P (121 mg, 0.46 mmol). The mixture was allowed to warm to room temperature for 3 hours, and then concentrated, purified by chromatography (EtOAc/hexane, 1:9) to afford **63** (122 mg, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.70 (1H, s), 7.78-7.34 (10H, m), 4.87 (1H, dd, J = 9.2, 10.4 Hz, H-3), 3.96 (1H, dd, J = 4.5, 11.5 Hz, H-1 eq), 3.90 –3.84 (2H, m, H-6), 3.44 (1H, t, J = 9.2 Hz, H-4), 3.42 (3H, s, OMe), 3.20 (1H, m, H-5), 3.14 (1H, t, J = 11.5 Hz, H-1 ax), 2.50-2.40 (2H, m, H-2, H-1′), 2.38 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.20 (1H, m, H-1′), 1.66 (2H, m), 1.40-1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7, 173.5, 135.8, 135.6, 133.7, 133.2, 129.6, 127.6, 127.5, 80.6, 78.2, 77.1, 69.0, 60.2, 42.4, 36.4, 34.4, 31.9, 29.6, 29.4, 29.3, 29.2, 26.8, 24.9, 22.6, 19.4, 14.1

2-C-(Carboxymethyl)-1, 5-anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl- 2-C-(2-propenyl)-D-glucitol **(64)**



To a solution of **63** (80 mg, 0.12 mmol) in *t*-BuOH (3 ml) and 2-methyl-2-butene (1 ml) was added a solution of $NaClO_2$ (110 mg, 1.23 mmol) and NaH_2PO_4 (169 mg, 1.23 mmol) in water (2 ml) slowly. The mixture was stirred for 1 hour, then diluted with ice-water (5 ml) and extracted with EtOAc (10 ml x 3). The combined organic extracts were washed with brine (5 ml), dried over Na_2SO_4, evaporated and further dried under high vacuum overnight to afford **64**. The acid was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.34 (10H, m), 4.90 (1H, dd, J = 9.2, 10.6 Hz, H-3), 4.05 (1H, dd, J = 4.6, 11.5 Hz, H-1 eq), 3.90 –3.86 (2H, m, H-6), 3.44 (1H, t, J = 9.2 Hz, H-4), 3.42 (3H, s, OMe), 3.22 (1H, m, H-5), 3.18 (1H, t, J = 11.5 Hz, H-1 ax), 2.40-2.30 (4H, m, H-2, H-1′, $OC(O)CH_2$), 2.18 (1H, m, H-1′), 1.66 (2H, m), 1.40-1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 173.5, 135.7, 135.5, 133.6, 133.2, 129.5, 127.6, 127.5, 80.6, 78.3, 76.8, 69.3, 63.1, 60.3, 38.6, 34.5, 32.7, 31.9, 29.7, 29.6, 29.4, 29.3, 26.9, 25.0, 22.8, 19.5, 14.2; HRMS calcd $C_{39}H_{60}O_7SiNa$ ($M+Na^+$) 691.4006, found 691.4002

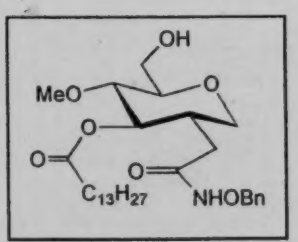
1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-6-O-tert-butyldiphenylsily-2-deoxy -4-O-methyl-3-O-myristoyl-D-glucitol **(65)**



To a solution of the acid **64** (25 mg, 37 µmol) and $BnONH_2$·HCl (7.2 mg, 0.04 mmol) in CH_2Cl_2 (0.3 ml) were added successively EDC (9.5 mg, 0.05 mmol) and triethylamine (10 µl, 0.08 mmol) at room temperature. The reaction mixture was stirred

for 2 hours. CH_2Cl_2 (10 ml) was then added and the solution was washed with water (5 ml) and brine (5 ml), then dried over Na_2SO_4. The residue after concentration was purified by chromatography (toluene/EtOAc, 4:1) to afford **65** (19 mg, 68 %).^{1}H NMR (400 MHz, CD_3OD) δ 7.78-7.34 (10H, m), 4.80 (3H, overlapped with H_2O, H-3, OCH_2Ph), 3.86 (2H, m, H-6), 3.84 (1H, dd, J = 4.5, 11.5 Hz, H-1 eq), 3.43 (1H, t, J = 9.4 Hz, H-4), 3.40 (3H, s, OMe), 3.20 (1H, m, H-5), 3.14 (1H, t, J = 11.5 Hz, H-1 ax), 2.42-2.34 (2H, m, $OC(O)CH_2$), 2.25 (1H, m, H-2), 2.06 (1H, dd, J = 5.1, 14.8 Hz, H-1'), 1.82 (1H, dd, J = 8.7, 14.8 Hz, H-1'), 1.63 (2H, m), 1.40-1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, J = 6.7 Hz) ;^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 170.2, 136.9, 136.7, 134.4, 130.8, 130.3, 129.6, 129.4, 128.7, 128.6, 81.9, 79.8, 79.0, 78.6, 70.2, 64.4, 60.8, 40.2, 35.3, 33.2, 32.6, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 27.5, 26.0, 23.9, 20.3, 14.6 ; HRMS calcd $C_{46}H_{67}NO_7SiNa$ ($M+Na^+$) 796.4584, found 796.4583

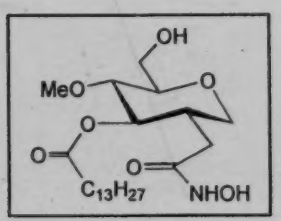
1, 5-Anhydro-2-deoxy-2-C-(carboxymethyl N-hydroxyamide)-4-O-methyl-3-O-myristoyl-D-glucitol **(66)**



A solution of **65** (16 mg, 21 μmol) in THF (0.3 ml) was treated with TBAF (1.0 M solution in THF, 0.10 ml, 0.10 mmol) at room temperature under Argon. After 2.5 hours, the reaction was concentrated. The residue was dissolved in EtOAc (10 ml) and then washed with water (5 ml x 2), brine, dried over Na_2SO_4 and evaporated. The residue was purified by chromatography (toluene/EtOAc, 1:1) to afford **66** (10 mg, 91%). ^{1}H NMR (400 MHz, CD_3OD: $CDCl_3$ = 3:1) δ 7.40-7.34 (5H, m), 4.80 (3H, overlapped

with H_2O, H-3, OCH_2Ph), 3.86 (1H, dd, J = 4.8, 11.7 Hz, H-1 eq), 3.77 (1H, dd, J = 1.8, 11.9 Hz, H-6), 3.63 (1H, dd, J = 4.3, 11.9 Hz, H-6), 3.41 (3H, s, OMe), 3.25-3.11 (3H, m, H-5, H-4, H-1 ax), 2.42-2.34 (2H, m, $OC(O)CH_2$), 2.26 (1H, m, H-2), 2.05 (1H, dd, J = 4.9, 14.8 Hz, H-1'), 1.80 (1H, dd, J = 8.9, 14.8 Hz, H-1'), 1.63 (2H, m), 1.40-1.20 (20H, m), 0.88 (3H, t, J = 6.7 Hz) ; ^{13}C NMR (100 MHz, CD_3OD: $CDCl_3$ = 3:1) δ175.3, 170.2, 136.8, 136.0, 130.5, 130.3, 129.9, 129.7, 129.5, 128.6, 81.8, 79.9, 79.1, 78.5, 70.3, 62.4, 60.9, 39.9, 35.4, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 27.3, 25.9, 23.8, 14.6; HRMS calcd for $C_{30}H_{49}NO_7Na$ ($M+Na^+$) 558.3407, found 558.3408

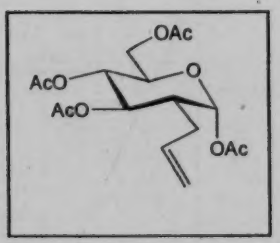
1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-4-O-methyl-3-O-myristoyl-D-glucitol **(5)**



Pd/C (8 mg) was added to a solution of **66** (10 mg, 19 μmol) in AcOH (2 ml). The reaction mixture was stirred for 2.5 hours under a balloon of H_2. The reaction mixture was then filtered through celite and the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **5** (5.3 mg, 64%). ^1H NMR (400 MHz, CD_3OD) δ 4.87 (1H, dd, J = 8.7, 10.8 Hz, H-3), 3.93 (1H, dd, J = 4.8, 11.6 Hz, H-1 eq), 3.79 (1H, dd, J = 2.0, 11.9 Hz, H-6), 3.65 (1H, dd, J = 4.2, 11.9 Hz, H-6), 3.41 (3H, s, OMe), 3.28 (3H, m, H-4, H-5, H-1ax), 2.48-2.36 (2H, m, $OC(O)CH_2$), 2.28 (1H, m, H-2), 2.10 (1H, J = 5.0, 14.5 Hz, H-1'), 1.84 (1H, dd, J = 9.0, 14.5 Hz, H-1'), 1.70 (2H, m), 1.40-1.30 (20H, m), 0.90 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.3,

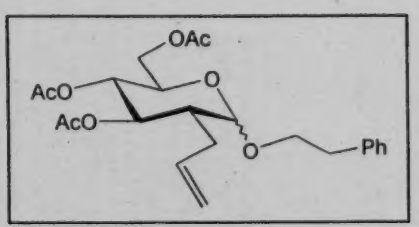
170.3, 81.9, 79.9, 78.5, 70.3, 62.3, 60.7, 40.0, 35.2, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 25.9, 23.7, 14.4; HRMS calcd for $C_{23}H_{43}NO_7Na$ ($M+Na^+$) 468.2937, found 468.2934

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-C-(2-propenyl)-α-D-glucopyranose **(67)**



To a solution of **21** (500 mg, 1.85 mmol) in Ac_2O (20 ml) was added TFA (2.0 ml) at 0 °C. The resulting slightly pink colored solution was stirred for 1 hour at the same temperature. The reaction mixture was then evaporated to dryness with toluene. The residue was purified by chromatography (EtOAc/hexane, 1:4) to afford **67** (688 mg, 100 %). ^1H NMR (400 MHz, $CDCl_3$) δ 6.10 (1H, d, J = 3.2 Hz, H-1), 5.62 (1H, m, H-2′), 5.22 (1H, dd, J = 9.4, 11.0 Hz, H-3), 5.00 (1H, t, J = 9.4 Hz, H-4), 4.99-4.92 (2H, m, H-3′), 4.25 (1H, dd, J = 4.7, 13.0 Hz, H-6), 4.00-3.95 (2H, m, H-5, H-6), 2.20-1.90(15H, m, 4OAc, H-2, 2H-1′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.4, 169.6, 168.7,133.9, 117.5, 91.6, 71.8, 69.7, 69.1, 61.9, 42.9, 31.6, 20.7, 20.6; HRMS calcd for $C_{17}H_{24}O_9Na$ ($M+Na^+$) 395.1318, found 395.1322

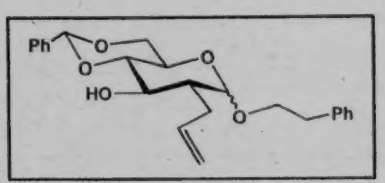
Phenylethyl 3,4,6-tri-O-acetyl-2-deoxy-2-C-(2-propenyl)-α/β-D-glucopyranoside **(68)**



To a solution of **67** (120 mg, 0.323 mmol), phenethyl alcohol (0.15 ml, 1.292 mmol) and 4 Å MS (100 mg) in CH_2Cl_2 (2.5 ml) was added $BF_3{\cdot}OEt_2$ (0.12 ml,

0.96 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. The mixture was filtered through Celite, and the Celite pad was washed well with CH_2Cl_2. The organic solution was washed with $NaHCO_3$ (5 ml), brine (5 ml), dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 3:1) to afford **68** (133 mg, 95%) as an inseparable 50:50 (α/β) anomeric mixture. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.16 (5H, m), 5.60 (1H, m, H-2'), 5.18 (0.5H, dd, J = 9.4, 10.9 Hz, H-3), 5.02 (0.5H, dd, J = 9.1, 11.1 Hz, H-3), 4.96-4.75 (3H, m), 4.72 (0.5 H, d, J = 3.3 Hz, H-1), 4.58 (0.5H, d, J = 8.9 Hz, H-1), 4.48 (0.5 H, m), 4.18-4.04 (1.5H), 3.92-3.78 (1H, m), 3.65-3.50 (2H, m), 2.95-2.84 (2H, m), 2.15-1.90 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.4, 170.3, 169.8, 138.8, 138.5, 134.9, 133.7, 128.9, 128.4, 128.3, 128.2, 126.3, 117.4, 116.8, 102.4, 98.3, 72.5, 72.4, 71.5, 70.7, 70.0, 69.7, 68.7, 67.6, 62.4, 62.2, 44.9, 44.1, 36.1, 36.0, 31.7, 30.6, 20.7, 20.6; HRMS calcd for $C_{23}H_{30}O_8Na$ ($M+Na^+$) 457.1838, found 457.1835

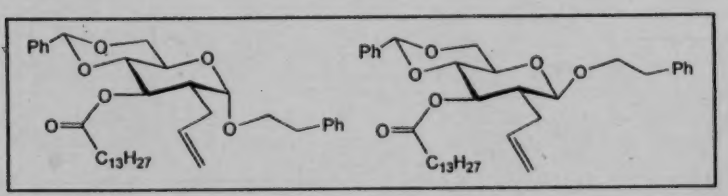
Phenylethyl 4,6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-α/β-D-glucopyranoside **(69)**



To a solution of **68** (52 mg, 0.12 mmol) in MeOH (3 ml), NaOMe (catalytic) was added at room temperature and stirred for 3 hours. The reaction mixture was then neutralized using Amberlite IR-120 (H). And then it was filtered and concentrated. The α/β anomeric mixture still could not be separated by chromatography (CH_2Cl_2/MeOH, 15:1). To a suspension of the triol in CH_3CN (2 ml), benzaldehyde dimethylacetal (30 µl, 0.24 mmol) and $TsOH{\cdot}H_2O$ (5 mg, 26 µmol) were successively added at room temperature. After 2 hours, triethylamine (100 µl) was added to the reaction mixture at 0

°C and the solvent was removed by evaporation. The residue was purified by chromatography (EtOAc/toluene, 1:9) to afford **69** (45 mg, 94%) as an α/β anomeric mixture. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (d, J = 3.5 Hz, H-1 α), 4.32 (d, J = 8.9 Hz β); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 138.5, 137.4, 137.2, 136.0, 134.5, 129.3, 129.2, 128.9, 128.5, 128.3, 126.3, 126.2, 117.5, 116.7, 102.9, 101.9, 99.2. 83.5, 82.7, 70.8, 70.3, 69.8, 69.2, 68.8, 68.7, 68.6, 66.0, 62.5, 46.9, 46.2, 36.2, 31.6, 30.3; HRMS calcd for $C_{24}H_{28}O_5Na$ ($M+Na^+$) 419.1834, found 419.1833

Phenylethyl 4,6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-3-O-myristoyl- α-D-glucopyranoside **(71)** *and Phenylethyl 4,6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-3-O-myristoyl-β-D-glucopyranoside* **(70)**



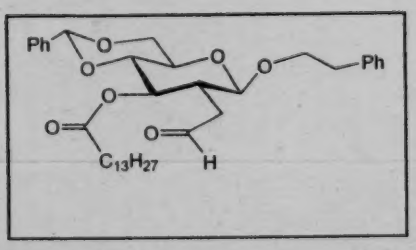
To a solution of **69** (135 mg, 0.34 mmol) in pyridine (5 ml), myristoyl chloride (0. 17 ml, 0.63 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 4 hours. EtOAc (20 ml) was then added to the reaction mixture and the solution washed with water (5 ml x 2), and brine (5 ml), dried over Na_2SO_4 and concentrated. Chromatography with (acetone/toluene, 1:60) gave the best separation of the anomeric mixture, **71** (α) 94 mg (45%) and **70** (β) 87 mg (42%).

70 β : ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.20 (10H, m), 5.74 (1H, m, H-2′), 5.48 (1H, s, CHPh), 5.20 (1H, dd, J = 9.3, 10.9 Hz, H-3), 4.97-4.78 (2H, m, H-3′), 4.38 (1H, d, J = 8.8 Hz, H-1), 4.28 (1H, dd, J = 4.9, 10.2 Hz, H-6), 4.13 (1H, dt, J =9.4, 6.4 Hz, OCH_2),

3.77 91H, t, J = 10.2 Hz, H-6), 3.64 (1H, m, OCH_2), 3.57 (1H, t, J = 9.3 Hz, H-4), 3.38 (1H, m, H-5), 2.92 (2H, t, J =6.4 Hz, CH_2Ph), 2.30 (2H, t, J = 7.3 Hz, $OC(O)CH_2$), 2.20 (1H, m, H-1′), 2.12 (1H, m, H-2), 1.92 (1H, m, H-1′), 1.59 (2H, m), 1.34-1.12 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 138.5, 137.2, 133.9, 128.9, 128.8, 128.3, 128.1, 126.3, 125.9, 117.5, 102.9, 101.3, 80.4, 70.8, 70.5, 68.8, 66.2, 45.7, 36.2, 34.3, 31.9, 30.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 25.1, 22.7, 14.1; HRMS calcd for $C_{38}H_{54}O_6Na$ ($M+Na^+$) 629.3818, found 629.3813

71 α : ^1H NMR (400 MHz, $CDCl_3$) δ 7.42-7.20 (10H, m), 5.57 (1H, m, H-2′), 5.47 (1H, s, CHPh), 5.33 (1H, dd, J = 9.5, 10.8 Hz, H-3), 4.93-4.77 (2H, m, H-3′), 4.71 (1H, d, J = 3.4 Hz, H-1), 4.16 (1H, dd, J = 4.5, 9.8 Hz, H-6), 3.88 (1H, dt, J = 9.4, 6.4 Hz, OCH_2), 3.77 (1H, m, H-5), 3.69 (1H, t, J = 9.8 Hz, H-6), 3.73 (1H, t, J = 9.5 Hz, H-4), 3.72 (1H, m, OCH_2), 3.57 (1H, t, J = 9.3 Hz, H-4), 3.38 (1H, m, H-5), 2.90 (2H, t, J = 6.4 Hz, CH_2Ph), 2.30 (2H, t, J = 7.3 Hz, $OC(O)CH_2$), 2.10 (2H, m, 2H-1′), 1.95 (1H, m, H-2), 1.59 (2H, m), 1.34-1.12 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 138.8, 137.3, 135.2, 128.9, 128.8, 128.4, 128.1, 126.3, 126.1, 101.4, 99.1, 80.9, 70.6, 69.1, 69.8, 63.0, 45.0, 36.1, 34.4, 31.7, 29.6, 29.5, 29.3, 29.2, 29.0, 25.1, 22.7, 14.1; HRMS calcd for $C_{38}H_{54}O_6Na$ ($M+Na^+$) 629.3818, found 629.3813

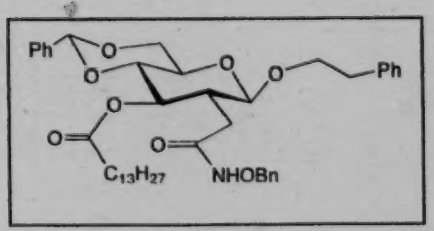
Phenylethyl 2-C-(aldehydomethyl)-4,6-O-benzylidene-2-deoxy-3-O-myristoyl- β-D-glucopyranoside **(72)**



Ozone was passed through a solution of **70** (87 mg, 0.14 mmol) in CH_2Cl_2 (10 ml) at –78 °C until the

solution turned blue. The excess ozone was removed with a stream of oxygen for 10 minutes, followed by addition of Ph_3P (75 mg, 0.29 mmol). The mixture was allowed to warm to room temperature for 3 hours. It was then concentrated and purified by chromatography (acetone/toluene, 1:30) to afford **72** (70 mg, 80%). ^1H NMR (500 MHz, $CDCl_3$) δ 9.48 (1H, s), 7.42-7.18 (10H, m), 5.48 (1H, s, CHPh), 5.12 (1H, t, J = 10.1 Hz, H-3), 4.48 (1H, d, J = 8.0 Hz, H-1), 4.31 (1H, dd, J = 4.9, 10.5 Hz, H-6), 4.11 (1H, dt, J = 10.4, 7.2 Hz, OCH_2), 3.79 (1H, t, J = 10.2 Hz, H-6), 3.65 (1H, m, OCH_2), 3.61 (1H, t, J = 9.3 Hz, H-4), 3.38 (1H, m, H-5), 2.86 (2H, t, J = 7.2 Hz, CH_2Ph), 2.38-2.24 (5H, m, $OC(O)CH_2$, 2H-1′, H-2), 1.56 (2H, m), 1.34-1.12 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7, 173.0, 138.1, 137.0, 128.9, 128.8, 128.4, 128.1, 126.4, 126.0, 103.1, 101.4, 78.0. 71.4, 70.9, 68.7, 66.6, 42.4, 41.4, 35.9, 34.2, 31.9, 30.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 25.1, 22.7, 14.1; HRMS calcd for $C_{37}H_{52}O_7Na$ ($M+Na^+$) 631.3611, found 631.3607

Phenylethyl 4,6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl- β*-D-glucopyranoside* **(74)**



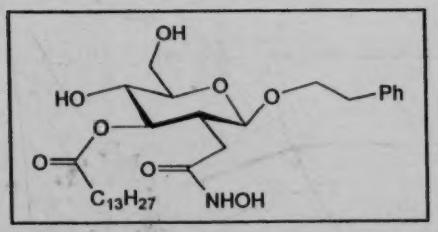
To a solution of **72** (62 mg, 0.10 mmol) in *t*-BuOH (2 ml), 2-methyl-2-butene (1 ml) and MeCN (1 ml) was added a solution of $NaClO_2$ (91mg, 1.02 mmol) and NaH_2PO_4 (141 mg, 1.02 mmol) in water (1.5 ml) slowly. The mixture was stirred for 1 hour, then diluted with ice-water (5 ml), and extracted with EtOAc (10 ml x 3). The combined organic extracts were washed with

brine (5 ml), dried over Na_2SO_4, evaporated and further dried under oil vacuum overnight to give the acid. The acid was used for the next step without purification.

To the crude acid and PyBOP (58.3 mg, 0.11 mmol) in DMF (0.3 ml) was added a drop of i-Pr_2EtN, followed by addition of a solution of $BnONH_2$·HCl (18 mg, 0.11 mmol) and i-Pr_2EtN (89 µl, 0.51 mmol) in DMF (0.4 ml). The reaction mixture was stirred at room temperature for 5 hours and then diluted with EtOAc (10 ml). The mixture was washed with water (2 x 5 ml), dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (EtOAc: toluene, 1/4) to afford **74** (58 mg, 80%) over two steps.

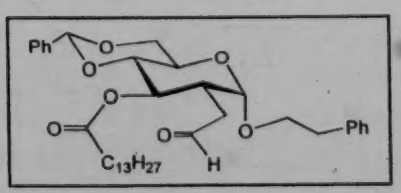
^{1}H NMR (500 MHz, CD_3OD: $CDCl_3$= 2:1) δ 7.42-7.14 (15H, m), 5.46 (1H, s, CHPh), 5.16 (1H, t, J = 10.7 Hz, H-3), 4.76 (2H, s, OCH_2Ph), 4.61 (1H, d, J = 8.6 Hz, H-1), 4.26 (1H, dd, J = 5.0, 10.4 Hz, H-6), 4.11 (1H, dt, J = 10.4, 7.2 Hz, OCH_2), 3.79 (1H, t, J = 10.4 Hz, H-6), 3.68 (1H, m, OCH_2), 3.62 (1H, t, J = 9.3 Hz, H-4), 3.48 (1H, m, H-5), 2.87 (2H, t, J = 7.2 Hz, CH_2Ph), 2.31 (2H, t, J = 7.2 Hz, $OC(O)CH_2$), 2.28 (1H, m, H-1′), 2.14-2.00 (2H, m, H-2, H-1′), 1.56 (2H, m), 1.34-1.12 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{13}C NMR (125 MHz, CD_3OD: $CDCl_3$= 2:1) δ 174.9, 170.5, 139.8, 138.7, 136.9, 130.2, 130.1, 130.0, 129.9, 129.6, 129.4, 127.3, 127.2, 104.4, 102.6, 81.6, 73.3, 71.9, 69.8, 67.7, 44.9, 37.2, 35.3, 33.2, 31.8, 30.9, 307, 30.6, 30.2, 26.3, 23.9, 14.8; HRMS calcd for $C_{44}H_{60}O_8NNa$ ($M+Na^+$) 730.4319, found 730.4321

Phenylethyl 4,6-O-benzylidene-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-myristoyl- β*-D-glucopyranoside* **(6)**



Pd-C (10 mg) was added to a solution of **74** (20 mg, 28 μmol) in AcOH (2 ml). The reaction mixture was stirred for 6 hours under a balloon of H_2. After filtration through celite, the celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 20:1) to give **6** (9.4 mg, 60%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.24-7.18 (5H, m), 4.88 (1H, dd, J = 8.9, 11.1 Hz, H-3), 4.22 (1H, d, J = 8.6 Hz, H-1), 4.09 (1H, dt, 10.4, 7.2 Hz, OCH_2), 3.85 (1H, dd, J = 2.3, 11.9 Hz, H-6), 3.72-3.66 (2H, m, H-6, OCH_2), 3.40 (1H, t, J = 11.1 Hz, H-4), 3.30 (1H, m, H-5), 2.90 (2H, t, J = 7.2 Hz, CH_2Ph), 2.37 (2H, m, $OC(O)CH_2$), 2.18 (1H, m, H-2), 2.10 (1H, dd, J = 5.9, 14.7 Hz, H-1′), 1.95 (1H, dd, J = 6.1, 14.7 Hz, H-1′), 1.62 (2H, m), 1.38-1.25 (20H, m), 0.90 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.6, 170.9, 140.2, 130.1, 129.3, 127.2, 103.9, 77.9, 77.5, 71.6, 70.6, 62.6,44.3, 37.1, 35.0, 33.1, 32.0, 30.8, 30.7, 30.6, 30.5, 30.3, 25.8, 23.7, 14.4; HRMS calcd for $C_{30}H_{49}O_8NNa$ ($M+Na^+$) 574.3356, found 574.3359

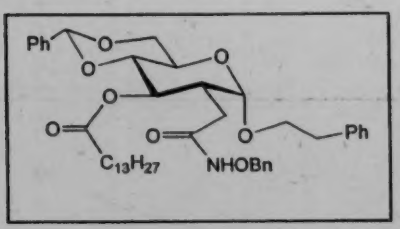
Phenylethyl 2-C-(aldehydomethyl)-4,6-O-benzylidene-2-deoxy-3-O-myristoyl- α -D-glucopyranoside **(73)**



Ozone was passed through a solution of **71** α (74 mg, 0.12 mmol) in CH_2Cl_2 (10 ml) at –78 °C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 minutes, followed by addition of Ph_3P (64 mg, 0.244

mmol). The mixture was allowed to warm to room temperature for 3 hours. It was then concentrated and purified by chromatography (acetone/toluene, 1:30) to afford **73** (69 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (1H, s), 7.40-7.18 (10H, m), 5.47 (1H, s, CHPh), 5.29 (1H, t, J = 10.1 Hz, H-3), 4.87 (1H, d, J = 3.1 Hz, H-1), 4.17 (1H, dd, J = 4.0, 10.3 Hz, H-6), 3.90 (1H, dt, J = 9.4, 6.4 Hz, OCH_2), 3.74 (1H, m, H-5), 3.70 (1H, t, J = 10.3 Hz, H-6), 3.57 (1H, t, J = 10.1 Hz, H-4), 3.52 (1H, m, OCH_2), 2.86 (2H, t, J = 6.4 Hz, CH_2Ph), 2.53-2.44 (2H, m, H-2, H-1′), 2.40 (1H, m, H-1′), 2.28 (2H, m, $OC(O)CH_2$), 1.57 (2H, m), 1.33-1.44 (20H, m), 0.88 (3H, t, J = 6.7 Hz); ^{1}H NMR (100 MHz, $CDCl_3$) δ 199.9, 173.1, 138.5, 137.1, 128.8, 128.4, 128.0, 126.3, 125.9, 101.4, 98.8, 80.5, 69.9, 68.9, 62.9, 41.9, 39.9, 35.9, 34.4, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 25.1, 22.8, 14.2

Phenylethyl 4,6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl- α -D-glucopyranoside **(75)**

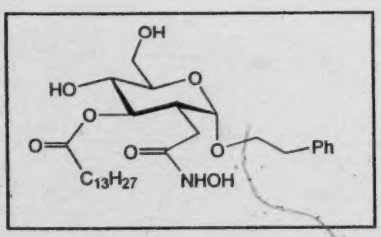


To a solution of **73** (51 mg, 84 μmol) in *t*-BuOH (2 ml), and 2-methyl-2-butene (1 ml) was added a solution of $NaClO_2$ (91 mg, 1.02 mmol) and NaH_2PO_4 (141 mg, 1.02 mmol) in water (1.5 ml) slowly. The mixture was stirred for 1 hour, then diluted with ice-water (5 ml), extracted with EtOAc (10 ml x 3). The combined organic extracts were washed with brine (5 ml), dried over Na_2SO_4, evaporated and further dried under oil vacuum overnight to afford the acid. The acid was used for the next step without purification. To the crude acid and PyBOP (48 mg, 0.09 mmol) in DMF (0.3 ml) was added a drop of i-Pr_2EtN, followed by addition of a

solution of $BnONH_2 \cdot HCl$ (15mg, 0.09 mmol) and *i*-Pr_2EtN (73 µl, 0.42 mmol) in DMF (0.4 ml). The reaction mixture was stirred at room temperature for 5 hours and then diluted with EtOAc (10 ml). The mixture was washed with water (2 x 5 ml), dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (EtOAc/toluene, 1:4) to afford **75** (49 mg, 82%) over two steps.

^1H NMR (400 MHz, CD_3OD: $CDCl_3$= 2:1) δ 7.40-7.16 (15H, m), 5.47 (1H, s, CHPh), 5.18 (1H, t, J = 9.5 Hz, H-3), 4.82 (1H, br s, H-1), 4.75 (2H, br s, OCH_2Ph), 4.03 (1H, dd, J = 4.8, 10.3 Hz, H-6), 3.80 (1H, m, OCH_2), 3.67 (1H, t, J = 10.3 Hz, H-6), 3.58 (1H, t, J = 9.5 Hz, H-4), 3.55-3.47 (2H, m, H-5, OCH_2), 2.86 (2H, t, J = 6.7 Hz, CH_2Ph), 2.45 (1H, m, H-2), 2.28 (2H, t, J = 7.3 Hz, $OC(O)CH_2$), 2.06 (1H, d, J = 7.1 Hz, 2H-1′), 1.54 (2H, m), 1.33-1.08 (20H, m), 0.86 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD: $CDCl_3$= 2:1) δ 175.4, 170.5, 140.2, 138.8, 136.8, 130.2, 130.1, 130.0, 129.8, 129.6, 129.2, 127.6, 127.4, 102.8, 100.2, 81.9, 79.1, 70.0, 64.4, 43.2, 37.1, 35.4, 33.2, 32.3, 30.9, 30.8, 30.6, 30.5, 30.1, 26.2, 23.9, 14.8; HRMS calcd for $C_{44}H_{60}O_8NNa$ ($M+Na^+$) 730.4319, found 730.4321

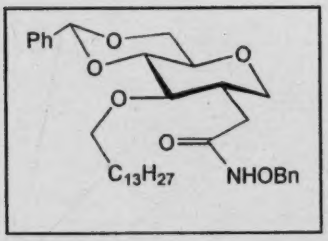
Phenylethyl 4,6-O-benzylidene-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-myristoyl- α -D-glucopyranoside (**7**)



Pd-C (10 mg) was added to a solution of **75** (15 mg, 21 µmol) in AcOH (10 ml). The reaction mixture was stirred for 6 hours under a balloon of H_2. After filtration through celite, the celite pad was washed

well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 20:1) to give **7** (7.2 mg, 62%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.30-7.18 (5H, m, Ph), 5.06 (1H, dd, J = 8.7, 11.3 Hz, H-3), 4.80 (1H, d, J = 3.2 Hz, H-1), 3.89 (1H, dt, J = 7.1, 9.6 Hz, OCH_2), 3.72 (1H, J = 2.1, 11. 8 Hz, H-6), 3.66-3.58 (2H, m, H-6 and OCH_2), 3.45 (1H, m, H-5), 3.42 (1H, t, J = 8. 7 Hz, H-4), 2.92 (2H, t, J = 7.1 Hz, CH_2Ph), 2.36 (2H, t, J = 7.4 Hz, $OC(O)CH_2$), 2.30 (1H, m, H-2), 2.05 (2H, m, 2H-1′), 1.62 (2H, m), 1.37-1.26 (20H, m), 0.90 (3H, t, J = 6.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 173.2, 140.4, 130.1, 129.4, 100.8, 75.8, 74.1, 71.0, 69.8, 62.5, 43.1, 37.0, 35.1, 33.1, 30.8, 30.7, 30.6, 30.5, 30.3, 25.9, 23.7, 14.2; HRMS calcd for $C_{30}H_{49}O_8NNa$ ($M+Na^+$) 574.3356, found 574.3359

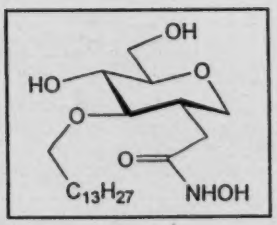
1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-tetradecane-D-glucitol **(79)**



Compound **17** (40 mg, 0.10 mmol) was dissolved in DMF (1 ml), sodium hydride (60% in oil, 10 mg, 0.25 mmol) and 1-bromotetradecane (89 µl, 0.30 mmol) were then added. The reaction mixture was stirred at room temperature for 5 hours. (TLC: EtOAc/toluene, 1:1 showed some starting material was still left). The mixture was diluted with CH_2Cl_2 (10 ml), and washed with diluted HCl (5 ml), sat. $NaHCO_3$, dried over Na_2SO_4, concentrated, the residue was purified by chromatography (EtOA/toluene, 3:1) to give **79** (40 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (1H, br s, NH), 7.50-7.30 (10H, m), 5.54 (1H, s, CHPh), 4.88 (2H, br s, OCH_2Ph), 4.27 (1H, dd, J = 4.9, 10.4

Hz, H-6), 4.01 (1H, dd, J = 4.9, 11.5 Hz, H-1 eq), 3.90 (1H, dt, J = 6.7, 9.3 Hz, OCH_2), 3.70 (1H, t, J =10.4 Hz, H-6), 3.56 (1H, t, J = 8.9 Hz, H-4), 3.48 (1H, m, OCH_2), 3.36-3.30 (2H, m, H-5, H-1ax), 3.26 (1H, dd, J = 8.9, 10.3 Hz, H-3), 2.40 (1H, br s, H-1′), 2.18 (1H, br s, H-2), 1.95 (1H, br s, H-1′), 1.50 (2H, m), 1.33-1.18 (20H, m), 0.89 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 137.5, 129.1, 128.8, 128.6, 128.1, 125.9, 101.1, 83.8, 80.5, 72.9, 71.7, 70.5, 68.9, 31.9, 30.2, 29.7, 29.6, 29.4, 29.3, 26.1, 22.6, 14.1; HRMS calcd for $C_{36}H_{54}NO_6$ ($M+H^+$) 596.3951, found 596.3953

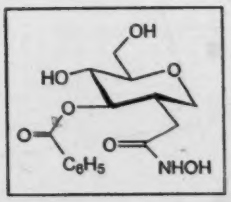
1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-tetradecane-D-glucitol **(8)**



Pd-C (20 mg) was added to a solution of **79** (30 mg, 52 μmol) in AcOH (2 ml). The reaction mixture was stirred for 6 hours under a balloon of H_2. After the filtration, the solvent was evaporated and the residue was purified on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **8** (13 mg, 70%). ^1H NMR (400 MHz, CD_3OD) δ 3.92-3.85 (2H, m, H-1 eq, OCH_2), 3.80 (1H, dd, J = 2.3, 11.8 Hz, H-6), 3.59 (1H, dd, J = 6.1, 11.8 Hz, H-6), 3.53 (1H, m, OCH_2), 3.33 (1H, d, J = 10.0 Hz, H-4), 3.14 (1H, t, J = 11.4 H-1ax), 3.12 (1H, m, H-5), 3.00 (1H, dd, J = 8.7, 10.0 Hz, H-3), 2.46 (1H, dd, J = 4.0, 14.3 Hz, H-1′), 2.05 (1H, m, H-2), 1.84 (H, dd, J = 9.5, 14.3 Hz, H-1′), 1.56 (2H, m), 1.40-1.20 (20H, m), 0.85 (3H, t, J = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 171.2, 85.9, 82.9, 73.8, 73.1, 70.5, 63.1, 33.1, 32.6, 31.4, 30.8, 30.7, 30.5, 27.2, 23.7, 14.4; HRMS calcd for $C_{22}H_{43}NO_6Na$ ($M+Na^+$) 440.2988, found 440.2990

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-benzyol-D-glucitol

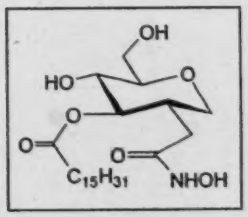
(9a)



^{1}H NMR (500 MHz, CD_3OD) δ 8.10-7.48 (5H, COC_6H_5), 5.02 (1H, dd, *J* = 9.0, 10.8 Hz, H-3), 4.03 (1H, dd, *J* = 4.7, 11.6 Hz, H-1eq), 3.85 (1H, dd, *J* = 2.4, 11.9 Hz, H-6), 3.67 (1H, dd, *J* = 5.7, 11.9 Hz, H-6), 3.58 (1H, t, *J* = 9.0 Hz, H-4), 3.30 (2H, m, H-5 and H-1ax), 2.42 (1H, m, H-2), 2.20 (1H, dd, *J* = 4.2, 14.6 Hz, H-1'), 1.91 (1H, dd, *J* = 9.8, 14.6 Hz, H-1'); HRMS calcd for $C_{15}H_{19}NO_7Na$ 348.1059; found 348.1057

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-palmitoyl-D-glucitol

(9b).



^{1}H NMR (400 MHz, CD_3OD) δ 4.75 (1H, dd, *J* = 9.0, 10.8 Hz, H-3), 3.94 (1H, dd, *J* = 4.7, 11.6 Hz, H-1eq), 3.80 (1H, dd, *J* = 2.3, 11.8 Hz, H-6), 3.62 (1H, dd, *J* = 5.7, 11.8 Hz, H-6), 3.38 (1H, t, *J* = 9.0 Hz, H-4), 3.25-3.15 (2H, m, H-5 and H-1ax), 2.38 (1H, t, *J* = 7.5 Hz, $OC(O)CH_2$), 2.25 (1H, m, H-2), 2.10 (1H, dd, *J* = 4.9, 14.5 Hz, H-1'), 1.82 (1H, dd, *J* = 9.1, 14.5 Hz, H-1'), 1.60 (2H, m), 1.40-1.20 (24H, m), 0.88 (3H, t, *J* = 6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 170.3, 82.7, 79.1, 70.7, 70.3, 62.9, 39.8, 35.1, 33.1, 32.7, 30.8, 30.7, 30.6, 30.4, 30.2, 25.9, 23.7, 14.4; HRMS calcd for $C_{24}H_{45}NO_7Na$ 482.3094; found 482.3097

Chapter 3

Inhibitor Evaluation

3.1 LpxC activity assay[1].

The *E. coli* LpxC substrate, [α-^{32}P]-UDP-3-O-(*R*-3-hydroxymyristoyl)-GlcNAc was prepared and purified as described previously by acylation of [α-^{32}P] UDP-GlcNAc using purified *E. coli* LpxA (provided by T. J. O. Wyckoff, Duke University) [26, 52]. These assays were performed at 30 °C, and contained 4 μM UDP-3-O-(*R*-3-hydroxymyristoyl)GlcNAc, 1 mg/mL BSA in 25 mM sodium phosphate, pH 7.4 and 0.1 nM purified *E. coil* LpxC. The activity assays were performed in plastic micro-centrifuge tubes in a reaction volume of 20 μL. At each time point (chosen so that the total conversion to product was less than 10%), 5 μL portions of each reaction mixture were removed and added to 1 μL of 1.25 M NaOH to stop the reaction. The alkaline samples were incubated for an additional 10 min at 30 °C to ensure complete hydrolysis of the ester-linked acyl chains from the LpxC substrate and product, and then were neutralized by the addition of 1 μL of 1.25 M acetic acid and 1 μL of 5% trichloroacetic acid. The neutralized samples were incubated on ice for 5 min and centrifuged for 2 min in a micro-centrifuge. Portions of the supernatants (1 μL) were spotted onto PEI-cellulose TLC plates for separation of the remaining substrate (detected as [α-^{32}P]-UDP-GlcNAc) from the product (detected as [α-^{32}P]-UDP-GlcN). After air drying, the plates were soaked for 10 min in methanol to improve resolution before chromatography. The plates were

[1] The assays were performed by Ms. Amanda L. McClerren in the laboratory of Prof. Christian R. H. Raetz in the Department of Biochemistry at Duke University, USA.

developed with 0.2 M aqueous guanidine-HCl as the solvent system. The radioactive spots on the plates were analyzed using a PhosphorImager equipped with ImageQuant software (Molecular Dynamics, Inc.) to determine the yields of product produced in each reaction mixture.

3.2 Inhibition of LpxC activity

Stock solutions (10 mg/mL) and any further dilutions of each inhibitor were made in 100% dimethylsulfoxide (DMSO). Compounds were added to a final concentration of 1 μg/mL to an assay mixture containing 4 μM M UDP-3-O-(*R*-3-hydroxymyristoyl)GlcNAc in 25 mM sodium phosphate buffer, pH 7.4. Additional DMSO was added to maintain compound solubility at a final assay concentration of 20% in DMSO. Purified *E. coli* LpxC was incubated with 1 mg/mL BSA in buffer and diluted into the final assay mixture to 0.1 nM at 30 °C. The initial velocities were plotted as a function of inhibitor concentration for each compound. The percent inhibition of each compound at 1μg/mL was calculated based on comparison of the specific activity of a reaction containing inhibitor to a wild-type reaction containing no inhibitor and substituting DMSO instead. The results are presented in Figure 3.1.

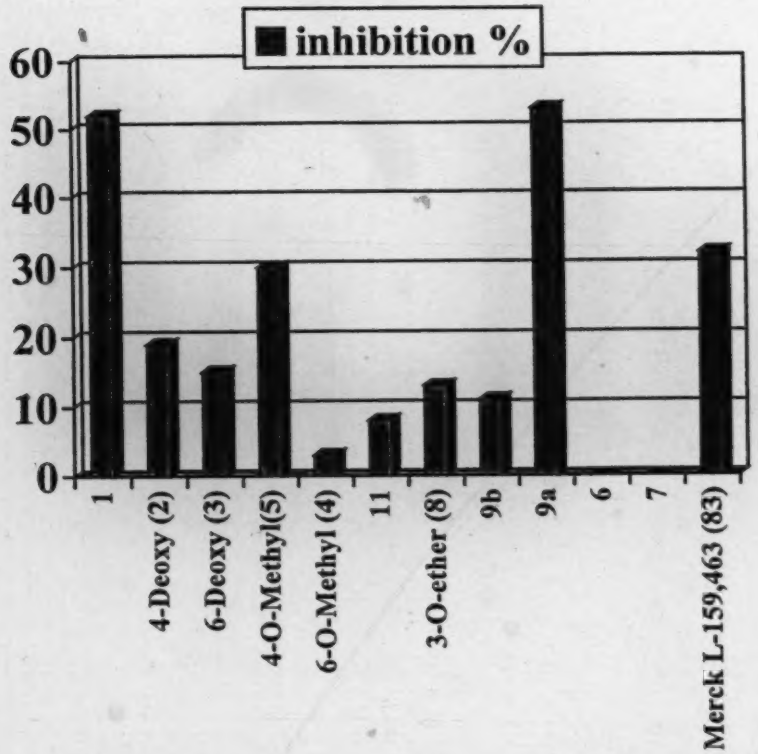


Figure 3.1. Inhibition[2] of all compounds at 1 μg/ml against *E. coli* LpxC

3.3 Interpretation of the structure-activity relationship

1. Both 4-OH and 6-OH are clearly involved in the binding interaction, since replacement of either hydroxyl group with hydrogen caused a loss in ΔG of 0.9 and 1.1 Kcal/mol, respectively, of the inhibitory activity (Table. 3.1).

2. The enzyme can not tolerate the steric bulk group at C-6, since the 6-O-methyl derivative had only barely detectable inhibitory activity (3%), while the enzyme can better tolerate the steric increase at C-4 to some extent (30%).

[2] approximate 10% error

Inhibitor	i	ΔΔ G (Kcal mol^{-1})	[I] (μM)
1	52%		2.32
4-Deoxy (**2**)	19%	-0.94	2.41
6-Deoxy (**3**)	15%	-1.12	2.41
11	8%	-1.59	2.61
4-O-Methyl (**5**)	30%	-0.54	2.24
6-O-Methyl (**6**)	3%	-2.14	2.24
3-O-ether (**8**)	13%	-1.21	2.39

Table 3.1. Binding energy change

The inhibition of **1** is competitive [38]. The estimated K_i can be calculated from the equation $i = [I]/([I] + K_i \{1 + [S]/K_m\})$, where i is the fractional inhibition, [I] the inhibitor concentration, and the [S] the substrate concentration [53].

$\Delta\Delta G = \Delta G_x - \Delta G_1$, $\Delta G = -RT \ln K_i$, where x and 1 refer to analog and parent inhibitor **1**, respectively, and $K_i = [E][I]/[EI]$.

3. Previous studies have shown that certain long acyl chains are crucial for the inhibition [38], which is confirmed here by the finding that cyclohexane derivative **11**, with an O-C_{14}-acyl group and a hydroxamic acid, maintained detectable inhibitory activity (8%). However, the carbohydrate derivative with a longer acyl chain (C_{16}) decreased the activity (11%). The reason may be proposed that the enzyme has a narrow cleft exactly designed to fit the myristoyl chain of the substrate (UDP-3-O-(*R*-3-hydroxymyristoyl)-GlcNAc) with the terminal methyl group packed against the enzyme

(Figure 3.2) and thus a longer acyl chain can not fit or would have to extend into solution via an open-ended tunel, which would be less favorable. The replacement of the acyl chain with a corresponding ether chain resulted in the loss of some activity. The reason for this could be that there is a hydrogen donor near the carbonyl group to form some interaction.

4. The native substrate contains a UDP moiety at the C-1 position, while the derivative with a phenylethyl group at such position (α and β) lost all inhibitory activity. This can be rationalized by two reasons. First the phenyl ring of this derivative is at the phosphate position rather than the position of the ribose ring of UDP which may create a *van der waals* clash. Secondly, to recognize a di-phosphate group, the surrounding should be polar as well. Therefore, replacing the phosphate group by hydrophobic moieties may not work well [54].

5. Replacement of the myristoyl ester chain with a benzoyl group maintained the same potent inhibitory activity.

In summary, a detailed picture of the topography of a substrate-based inhibitor binding with the LpxC enzyme can be proposed as shown in Figure 3.2. [55]

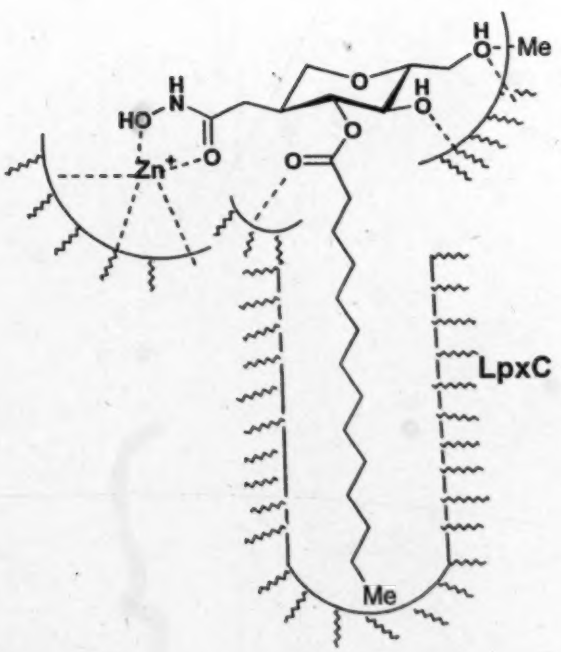


Figure 3.2. Schematic presentation of binding sites deduced by chemical mapping

3.4 Future work of the project

Having now probed the active site of this enzyme with these derivatives, it is clear that in order for any compound to be a more potent inhibitor of this enzyme the modification should be done at the OH-3 position. A library at C-3 with various acyl aromatic groups may lead to useful antibiotics.

Chapter 4

NMR studies on *A. aeolicus* LpxC in complex with substrate-based inhibitor 1[3]

The structural characterization of the LpxC enzyme by X-ray crystallography or NMR would facilitate the design of more potent and broad-spectrum deacetylase inhibitors useful as antibiotics. Due to the size and thermal stability, the 32 kDa, 282 residue LpxC from the hyperthermophlic organism *A. aeolicus* was particularly well suited for NMR structural studies. These studies were performed by the research team of Prof. Pei Zhou at Duke University.

Both free LpxC and LpxC in complex with substrate-based inhibitor NMR spectra have been recorded. Probably due to conformational mobility, some portions of the molecule could not be defined due to severe line broadening. The outcome was that the ^{1}H-^{15}N HSQC NMR spectra of LpxC did not show the expected number of crosspeaks in all conditions tested, so the structure could not be solved.

However the spectrum of the complex with inhibitor **1** showed noticeably more crosspeaks than that of the free protein and many crosspeaks showed significant perturbation, suggesting that the presence of inhibitor stabilizes key recognition elements in LpxC. Also the titration experiments (a series of ^{1}H-^{15}N HSQC spectra at 50 °C in the presence of an increasing molar ratio of the substrate-based inhibitor) reveal that the

[3] The structural determination of the LpxC /inhibitor **1** complex in solution was solved by Prof. Pei Zhou in the Department of Biochemistry at Duke University, USA

inhibitor binds to LpxC with a 1:1 molar ratio and that the rate of binding exchange is slow on NMR time scale. Base on these results, the solution structure of *A. aeolicus* LpxC in complex with inhibitor has been determined (Figure 4.1).

Figure 4.1. The solution structure of LpxC in complex with **1.** Ribbon representation of the structure. Domains I and II are colored green and light yellow, respectively, and the insert regions from domains I and II are colored pink and blue, respectively; linkers between the domains are colored in gray. Inhibitor **1** is shown as a spacefilling model with CPK coloring, and the zinc ion is shown as a spacefilling model beside it.

4.1 LpxC possesses a novel four-layered α/β fold

LpxC contains two similar domains, each possessing two layers of secondary structural elements, with a layer composed of two long alpha helices packing against a primary beta sheet (Figure 4.1). Domain I begins at the N-terminus and extends to residue 117; following a six residue inter-domain linker, domain II begins at residue 124 and continues to residue 268. The C-terminal fourteen residues of the protein are unstructured. The two domains pack against each other such that the alpha helices are on the interior and form the domain interface, and the beta sheets are on the exterior of the protein. The two domains and their insert components are colored differently in the figures to assist with the identification of the components.

The main beta sheets are each composed of five nearly identical strands. Proceeding along the primary sequence from the N-terminal end of each domain, one encounters four of the beta strands immediately, positioned antiparallel to each other, joined by short loops or tight turns. The fifth beta strand of the motif (β5 in domain I and β6' in domain II) is inserted between the second and third, antiparallel to the second and parallel to the third, to form a sheet with mixed parallel and antiparallel strands. The beta sheet of domain I is severely distorted, twisting about 80°, whereas that of domain II is essentially flat.

The long helices are each nine residues long in domain I (α1 and α2), and 14-15 residues long in domain II (α1' and α2'). Domain I features one helix (α1) which packs

against β5 at an angle of about 30°, and another (α2) which is nearly parallel to β1, leaving a pocket between them which is lined with hydrophobic residues; in domain II, these helices (α1' and α2') have parallel axes, and pack at an angle to cross three beta strands each. The helices and beta sheet of each domain form a distinct αα/βββββ structural motif. The gap at the bottom of each domain is closed with a small beta sheet (strands β0 and β6 for domain I, and strands β0' and β5' for domain II).

The insert regions (colored pink and blue in the figures) differ substantially between the domains. Domain I features a small, three-stranded, antiparallel beta sheet (βa, βb and βc; pink in the figures) with βa packing parallel to α2', whereas the domain II insert (blue in the figures) features a β/α/α $_L$/β motif with two parallel beta strands (βa' and βd') connected by a helix (αb') and a helical loop (α_Lc'). The insert is followed by a helical turn (α_Le') connecting to α2. This distinct motif (β/α/α_L/β) forms a closed loop with a hydrophobic passage, which is important in inhibitor binding. Both inserts are approximately perpendicular to the main beta sheets.

The two domains come together with their beta strands nearly parallel, and α1' of domain II intersecting α1 of domain I at about a 70° angle; a short helix (α0') from domain II is positioned next to α1' and α2, forming a structure resembling a four helix bundle (comprised of α1, α2, α0' and α1'), to seal the gap between the two domains. The two insert regions are on the same side of the molecule and together form the active site, in a cleft between the β/β/β and the β/α/α $_L$/β motifs, with access to the accessory pocket

between the two helices of domain I; the linker between the domains is located on the opposite side of the molecule.

An analysis of the LpxC structure by the DALI server was unable to find any known structure with a similar fold [56].

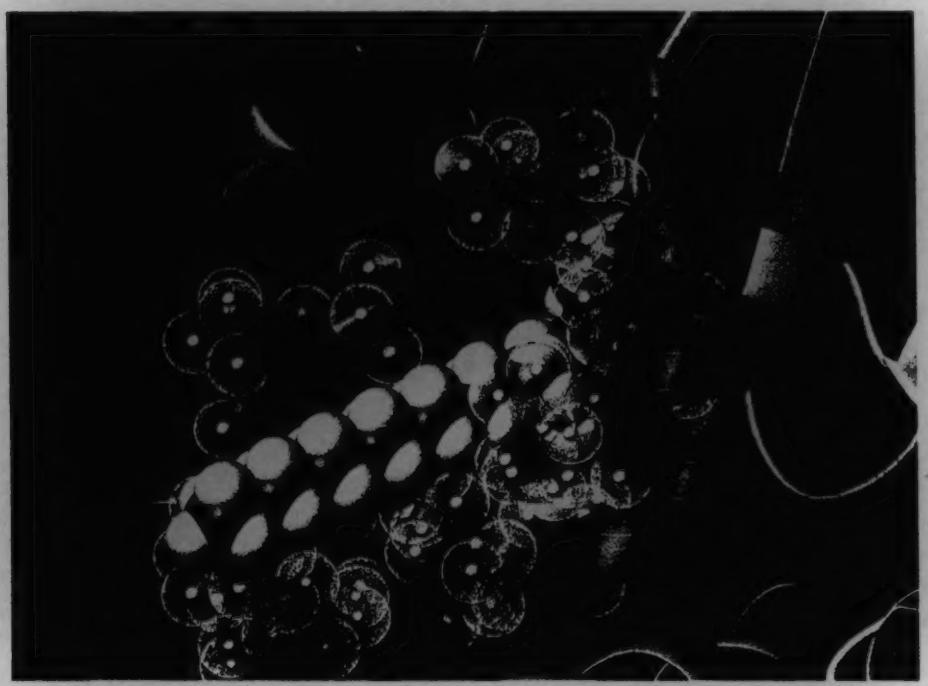


Figure 4.2. The **1** acyl chain binds in a hydrophobic passage. A spatial representation of the interaction, with the residues that define the passage shown as ball-and-stick models with *van der Waals* dot surfaces, and with **1** shown as a spacefilling model. The ribbons are colored by domain as in Figures 4.1.

4.2. LpxC captures 1 with a hydrophobic passage

The structure of the complex reveals that LpxC binds to **1** via the hydrophobic passage formed within the closed loop of the β/α/α ʟ/β motif of domain II, with the hexose ring buried, and the acyl chain extending out through the passage into solution. Figure 4.2 shows a close-up view of the acyl chain bound in the passage. The acyl chain contacts numerous hydrophobic sidechain atoms in residues T179, I186, I189, L200, T203, V205 and Y212, and these strong interactions provide an excellent explanation for the ability of LpxC to capture its substrate with high affinity, as well as its >5 x 10^6-fold lower activity with substrates lacking an acyl chain [26].

The positioning of the **1** acyl chain within the passage is well determined from numerous intermolecular NOE crosspeaks. Although the **1** spectrum shows a high degree of degeneracy among acyl chain resonances (methylene groups 6-11), signals from the terminal methyl, the penultimate methylene, and the α- and β-methylenes are well dispersed. The aromatic ring of Y212 and a methyl group of L200 both form *van der Waals* contacts with the terminal methyl of the acyl chain of **1**, while the alpha protons of the acyl chain are in close proximity to the methyl group of T179. The average distance between T179 and Y212 is approximately 15 Å, consistent with an extended conformation for the acyl chain of **1** from the α-methylene to the terminal methyl group. Although the binding of the acyl chain is clearly important, as UDP-GlcNAc is a poor substrate, it is not clear whether the recognition of a full length acyl chain is required since LpxC is unable to distinguish between acyl chains longer than C_{10} [57, 58].

The hexose ring of **1** adopts a chair conformation and sits in a largely enclosed pocket, on the interior side of the $\beta/\alpha/\alpha_L/\beta$ motif and beneath the $\beta/\beta/\beta$ motif, and binds through both polar and hydrophobic interactions. The backbone amide and the beta-hydroxyl group of T71, as well as the beta-hydroxyl group of S59, are positioned to form an intricate hydrogen bond network to recognize O5 and O6 of the sugar ring in **1**. T179 plays a pivotal role in supporting the hexose ring at its junction with the acyl chain, and displays NOE crosspeaks with H2 of the ring, the beta protons of the acyl chain, and the hydroxamate α-methylene. The hydroxamate terminal oxygen is positioned to form a hydrogen bond to the sidechain amine group of K227. The summary of the inhibitor-enzyme complex is shown in Figure 4.3.

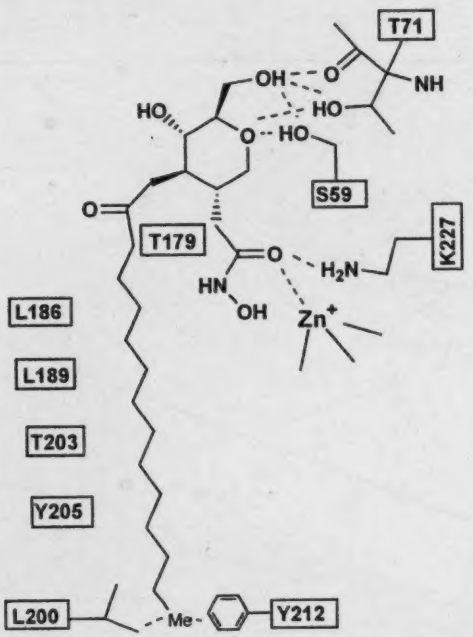


Figure 4.3. Hydrogen bond map for the complex

4.3 Methods

Expression and Purification of the Protein and Preparation of Samples. *A. aeolicus* LpxC was overexpressed in BL21(DE3)STAR cells (Invitrogen) grown in M9 minimal medium from the pET21 vector pAaLpxC [59]. Isotope enriched protein was prepared by using ^{13}C-glucose and $^{15}NH_4Cl$ (Cambridge Isotope Laboratory) as the sole carbon and nitrogen sources. Deuterium labeling was accomplished by growing the cells in M9 medium with 100% D_2O and deuterated ^{13}C-glucose (Cambridge Isotope Laboratory). Samples were prepared with [*U*-^{15}N], [*U*-^{13}C/^{15}N], [*U*-^{2}H/^{13}C/^{15}N], [50%-^{2}H, *U*-^{13}C/^{15}N] and [10%-^{13}C, *U*-^{15}N] labeling. A selective labeling protocol was employed to prepare samples labeled only with ^{15}N-Val, Ile, Leu, Lys, Phe or Tyr [60]. In addition, a uniformly ^{2}H/^{13}C/^{15}N-labeled, VIL-methyl protonated sample was prepared according to a previously described protocol [61]. LpxC was purified using anion-exchange (Q-Sepharose Fast Flow, Amersham) and size-exclusion (Sephacryl S-200 HR, Amersham) chromatography. The LpxC/**1** complex was prepared by adding **1** to the purified LpxC protein in a roughly equimolar amount and allowing the complex to form at room temperature over one hour. Samples were concentrated and buffer-exchanged into 25 m*M* sodium phosphate, pH 6.5, with 150 m*M* KCl, 4 m*M* dithiothreitol, 5% D_2O and 5% deuterated DMSO. An estimated 10-25% molar excess of **1** was then added to all samples of the complex, except for the samples used to record the F1/F2 ^{15}N/^{13}C-filtered 2D NOESY experiment, which were subjected to extensive dialysis to remove **1** that was not bound to LpxC. NMR samples contained 0.3-0.5 m*M* LpxC.

Titration of LpxC with 1. The titration experiment was conducted by mixing a sample of ^{15}N-labeled LpxC with increasing quantities of **1**. ^{1}H-^{15}N HSQC spectra were collected at **1**:LpxC molar ratios of 0:1, 1:3, 2:3, 1:1, 2:1 and 3:1. To exclude the effects of DMSO interactions with LpxC, a control HSQC spectrum was recorded of another sample of LpxC, mixed with 5% DMSO.

NMR Spectroscopy. Experiments were conducted at 50°C using Varian INOVA 600 and 800 MHz spectrometers. Data were processed using FELIX (Accelrys) and analyzed with XEASY.[30] HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB and HNCO experiments, along with ^{1}H-^{15}N-HSQC of selectively labeled samples, were used for sequential assignment, together with the PACES program, confirmed by manual analysis [62]. Sidechain resonances were assigned using 3D ^{15}N-TOCSY-HSQC, HN(COCA)HA and 3D HCCH-TOCSY experiments. Additional assignments were obtained through an H(CCO)NH-TOCSY experiment recorded on the [50%-^{2}H, *U*-^{13}C/^{15}N]-labeled sample [63]. Aromatic resonances were assigned based on 2D-homonuclear NOESY, 2D-homonuclear TOCSY and ^{13}C-aromatic NOESY-HSQC experiments. Stereospecific assignment of valine and leucine methyl groups was accomplished using a ^{1}H-^{13}C HSQC spectrum of the sample labeled with 10% ^{13}C. Resonances of **1** were assigned using F1/F2 ^{15}N/^{13}C-filtered 2D NOESY experiments recorded with a perdeuterated, ^{15}N/^{13}C labeled protein sample of the complex.

Structure Calculation. Distance constraints for LpxC were derived from 3D ^{15}N-NOESY-HSQC, 3D ^{13}C-NOESY-HSQC and homonuclear 2D NOESY with mixing

times of 60 ms, 80 ms, and 50 ms, respectively [64]. Distance constraints obtained from these experiments were autocalibrated using the CALIBA module included in the DYANA package. Loose constraints were also derived from methyl-methyl NOE experiments with a mixing time of 175 ms using a uniformly $^{2}H/^{13}C/^{15}N$ labeled and VIL-methyl-proton-labeled sample. Dihedral angle restraints were derived from TALOS analysis of the chemical shift information and from the HABAS routine based on the analysis of NOE patterns. Two constraints per hydrogen-bond ($d_{HN-O} \leq 2.5$ Å and $d_{N-O} \leq 3.5$ Å) were added for those amide protons that were protected from solvent exchange. Intermolecular NOEs between LpxC and **1** were detected through 3D F1-^{13}C-filtered, F3-edited ^{13}C-NOESY-HSQC (100 ms mixing time), 3D F1-^{13}C-filtered, F3-edited ^{13}C-aromatic NOESY-HSQC (100 ms mixing time) using a ^{13}C labeled sample or 3D F1-$^{13}C/^{15}N$-filtered, F3-edited ^{15}N-NOESY-HSQC (200 ms mixing time) experiments with a $^{2}H/^{13}C/^{15}N$ uniformly labeled complex. Intermolecular NOEs were classified into three classes—strong (≤ 3.0 Å) , medium (≤ 4.0 Å) and weak (≤ 5.0 Å)—for conversion into distance constraints. Initial structures were generated by using DYANA starting from random conformations and refined using the simulated annealing protocol in CNS. The 15 structures with no NOE violations larger than 0.4 Å and no dihedral angle violations larger than 4° were reported. [65]

Data Deposition. The chemical shifts for LpxC and **1** have been deposited with the BioMagResBank (accession number 5627), and the atomic coordinates for the complex have been deposited with the Protein Data Bank (accession number 1NZT, RCSB018397).

Chapter 5

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